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PROCEEDINGS

of the

ASSOCIATION FOR RESEARCH IN OPHTHALMOLOGY, INC.

SECTION MEETINGS

1958-1959

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RETINAL CIRCULATION TIME WITH THE AID OF FUNDUS CINEPHOTOGRAPHY*

MILTON FLOCKS, M.D., JOHN MILLER, M.D., AND PETER CHAO, M.D.
San Francisco, California

In 1957, Chao and Flocks[1] described a method of determining the circulation time of the cat retina using intracarotid artery injections of trypan blue, an ophthalmoscope, and a stop watch. With their technique they found the cat retinal circulation time to be approximately two seconds. They suggested that, if more innocuous indicator solutions could be used, the method could possibly be adapted for use in human beings.

If a cobalt-blue filter is inserted in the ophthalmoscope, the retina appears dark purple in color and the vessels black. When intravenously injected fluorescein reaches the central retinal artery, it lights up the artery to a fluorescent yellow if viewed through the cobalt-blue filter. However, the end-points, the exact moment when the fluorescein enters the retina via branches of the central retinal artery and the exact moment when the fluorescein leaves the retina as it crosses the disc margin via branches of the central retinal vein, are not as sharp as when trypan blue is used in the carotid artery. Consequently, when using the intravenous fluorescein technique with the ophthalmoscope, the circulation time readings are not as accurate.

The idea was conceived that the end-points could be ascertained more precisely by means of motion-picture photography using a motion-picture film viewer and editor to examine leisurely the individual frames of the film. With this technique the unavoidable error due to the reaction time of the person pressing the stop watch would be eliminated and, in addition, a permanent objective record of the circulation time and of the retinal circulation would be obtained.

MATERIALS AND METHODS

Pavia[2] first adapted a Zeiss-Nordenson fundus camera for moving pictures in 1933. For the present experiment a Cine Special 16-mm. movie camera was adapted to fit a Zeiss-Nordenson fundus camera.

The still-film housing was removed from the focusing tube of the fundus camera. A fitting containing a 20-diopter lens and a Wratten-2B filter was then fixed in this tube. The lens was necessary to reduce the image to 16-mm. film, thus retaining the same field as had previously been obtained on 828 film. The Wratten-2B filter as indicated by Tredinnick[3] was found useful as an ultraviolet-absorbing filter.

The table for the fundus camera was modified to hold separate mounts for the Cine Special and fundus camera. The Cine Special was mounted without a lens. A rubber tube fastened at the lens mount of the camera fit over the focusing tube of the fundus camera. Focusing was then easily effected by adjusting the tube while viewing through the reflex window of the Cine Special.

The carbon arc light of the fundus camera was filtered by a cobalt-blue filter (found by trial and error to be satisfactory).

A 16-mm. daylight Super Anscochrome film was used and processed at an emulsion speed of 100. A speed of 20 frames per second was found to give the best exposure.

Adult cats were anesthetized with 120 mg. of intraperitoneal nembutal. Their pupils were dilated with five-percent atropine. A cut down was performed on the femoral vein

* From the Division of Ophthalmology, Department of Surgery, Stanford University School of Medicine. This investigation was supported by Research Grant B-873 (C30), United States Public Health Service. Presented in part before the Western Section, on November 18, 1958, at San Francisco.

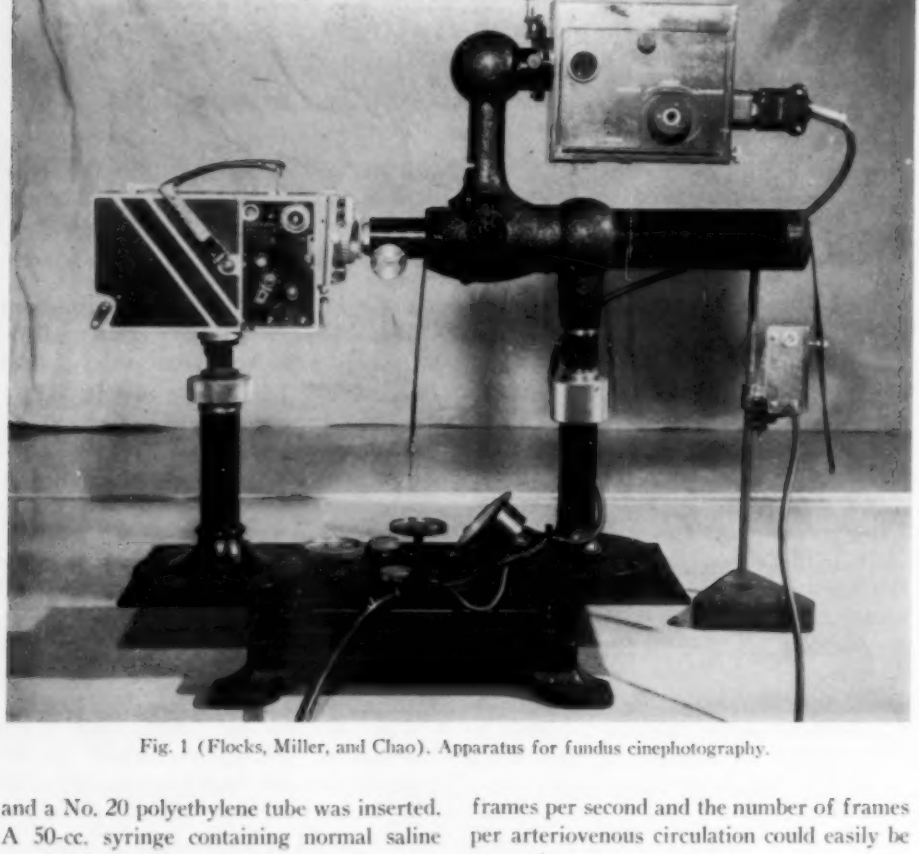

Fig. 1 (Flocks, Miller, and Chao). Apparatus for fundus cinephotography.

and a No. 20 polyethylene tube was inserted. A 50-cc. syringe containing normal saline was connected by way of a three-way stopcock to keep the cut down open.

The arcs were started and when the cat was positioned and the image of the fundus was focused in the viewer of the Cine Special, the camera was started, and two cc. of 10-percent sodium fluorescein was injected rapidly through the cut down.

Timing was done by a paddle six degrees in width turned by a 30 rpm synchronous motor which intersected the light beam between cat and camera one time per second. This marked the movie film at one-second intervals.

Using a 16-mm. movie editor with an attached frame counter the number of frames per second and the number of frames per arteriovenous circulation could easily be counted.

Results

The fluorescein coursing through the retinal arterioles and then entering the veins makes a beautiful motion picture which allows the dynamics of the retinal circulation to be studied at leisure.

Six of the 10 cats studied with fluorescein had pictures clear enough so that the circulation and the circulation times could be studied well. In four cats the pictures were inadequate because of poor focus or insufficient light. The circulation time of five of the six cats varied between 1.16 and 2.0 seconds. The other cat (No. 10) done twice with the same anesthesia had a circulation time of 3.3

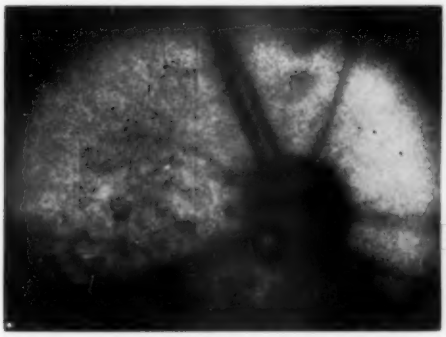

Fig. 2 (Flocks, Miller, and Chao). Cat retina prior to injection.

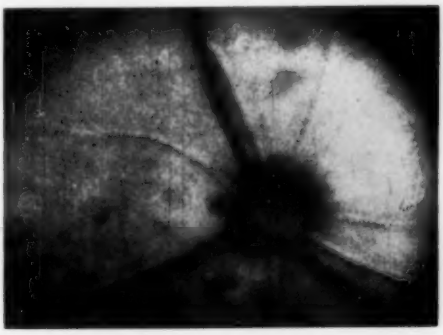

Fig. 3 (Flocks, Miller, and Chao). Fluorescein has entered the retinal arterioles.

seconds the first time and 0.9 seconds the second.

Cat Number	Circulation
1	1.28
2	2.00
3	1.16
4	1.52
6	1.80
10	3.33 (and 0.9)

Circulation times using intracarotid injection of trypan blue were photographed on several occasions. It was found that the circulation time could be increased by more forceful injection into the artery.

Two attempts to determine the retinal circulation times in human beings using motion pictures were unsuccessful because of insufficient light. Motion pictures of the retina of cats are much easier to make than motion pictures of the human retina. We are currently trying to improve the optical system of our apparatus so that this method can be applied to the study of the human retinal circulation time.

Comment

The large spread of circulation times using this method in comparison to the relatively uniform circulation times obtained by intracarotid injection of trypan blue method is interesting. We can speculate that it reflects physiologic variations in circulation which are not obscured by the intravenous route of injection as they might be by the intracarotid route where injection would directly alter arterial pressure. Continuing studies should further elucidate this point.

As this technique of studying the retinal circulation is further developed it may prove to be an invaluable tool for the study of the effect of drug changes in intraocular pressure and of retinal disease on the retinal circulation.

Summary

A technique of determining the retinal circulation time using intravenous fluorescein and fundus cinephotography is described. The results indicate that the retinal circulation time of cats is somewhat faster and

Fig. 4 (Flocks, Miller, and Chao). Fluorescein returning in retinal veins.

not as uniform as formerly thought. The motion picture technique affords a permanent objective record of the animal's retinal circulation which can be studied at leisure.

Clay and Webster Streets (15).

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STIMULUS ORIENTATION AND THRESHOLD: AN OPTICAL ANALYSIS*

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An old and puzzling problem of visual acuity studies is raised anew in a recent article by Ogilvie and Taylor (1958) who report that the threshold for very fine wires is lower in the vertical and horizontal than in the oblique orientation. They state that the analysis of variance indicates significant differences not only among the various directions of the wire but also among the five eyes tested. This orientation preference is now well established, having been found by a number of workers using different techniques. Ogilvie and Taylor cite six papers, which cite many others and I have twice encountered similar results in experimental work. A satisfactory explanation has, however, proved difficult. Since most workers have taken the obvious precaution to exclude eyes with "clinical" astigmatism, some have been forced, rather unhappily, to conclude that it is not an optical phenomenon. Thus Leibowitz (1953) suggests ". . . that meridional differences originate in part from factors of retinal or post-retinal origin." Possible nonoptical explanations, however, have been vague and not particularly convincing, as, for example, some sort of "grain" in the retinal organization ("retinal astigmatism" of Shlaer, 1937) or the supposed psychologic effect of the greater prevalence of vertical and horizontal lines in nature.

ORIENTATION PREFERENCE AND DIFFICULTY OF OBSERVATION

Additional proof of the occurrence of orientation preference is unnecessary but unpublished data obtained by me and a former student, Oscar C. Railsback, M.D.,† throw some light on the manner in which the preference arises. A number of observations were made on a small photographically reproduced line, or rather narrow rectangle, which at 7.5 meters subtended 2.11 by 0.55 minutes of arc. This was exposed in four orientations at distances up to 10 meters and answers required on the direction of the line.

The numbers of correct and incorrect answers are given in Table 1. In Figure 1 are plotted as a function of the angular length of the stimulus the percentage of correct answers for all orientations and the percentage of correct answers for the vertical position only.

The correct answers of course increase with the increase in the angular length of the stimulus; it will be noted that the percentage for the vertical line is slightly but not significantly greater than that for all

* From the Los Angeles College of Optometry. Presented before the Western Section, November, 1958, at San Francisco.

† I wish to thank Dr. Railsback, now a member of the Woodland Clinic Medical Group, Woodland, California, for the use of a report prepared over 30 years ago, yet so well organized and presented that the data were readily analyzed for the present purpose. I trust that this is some compensation for the frustration we then experienced in trying to interpret the results.

TABLE 1

NUMBERS OF CORRECT AND INCORRECT ANSWERS

Distance (m.)	Angular Length (min.)	Orientation				Sum of Correct	No. of Incorrect	Total
		Left Oblique	Vertical	Right Oblique	Horizontal			
7.5	2.108	44	53	51	49	197	23	220
8.0	1.977	41	51	48	37	177	43	220
8.5	1.860	37	49	34	25	145	75	220
9.0	1.757	20	38	25	22	105	115	220
9.5	1.664	18	40	17	16	91	129	220
10.0	1.581	6	40	6	4	56	164	220

answers at the largest size of the stimulus, but that for the smaller stimuli the percentage is markedly and significantly greater than that for all positions. The chi-square test indicates that the distribution of answers among the different positions for the largest stimulus could have happened by chance about three times in 100 but for the smaller stimuli less than once in 1,000 times. It will be noted that in the present case the preference is for the vertical alone and that the horizontal is not different from the obliques.

The greater preference for the vertical position in the more difficult task is still more strikingly shown in Figure 2. Here the number of correct answers for the vertical as a percentage of all correct answers is plotted as a function of the angular size of the stimulus in minutes. It will be seen that for the stimulus easiest to recognize, the number of correct answers for the vertical is approximately the 25 percent to be expected for one position in four, whereas for the stimulus most difficult to recognize, 72 percent were vertical. The chi-square test shows that this distribution of answers between the vertical and all other positions might be expected by chance less often than once in 1,000 times.

It is clear that in some manner the preference, in the present case for the vertical alone, is linked with the difficulty of the observation. This is in agreement with the results of other workers although none seems to have commented on this phase. Thus in Figure 1 of Ogilvie and Taylor it may be noted that the preference for the vertical

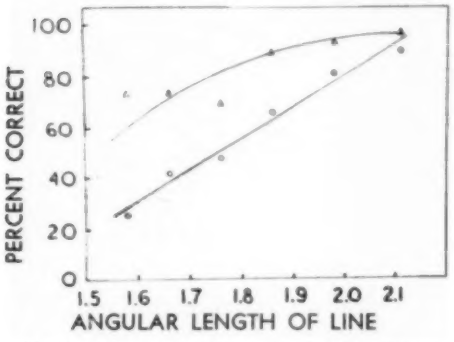

Fig. 1 (Weymouth). Percentages of correct answers as a function of the angular length of the target line in minutes. (Open circles, all orientations; triangles, vertical orientation only.)

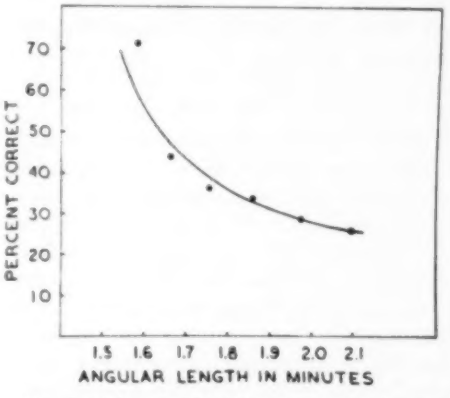

Fig. 2 (Weymouth). Correct answers for the vertical orientation as a percentage of all correct answers plotted as a function of the angular length of the target line in minutes.

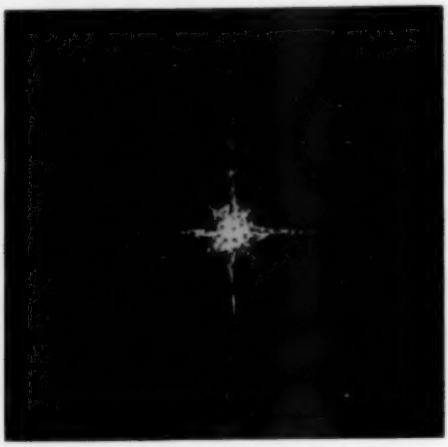

Fig. 3 (Weymouth). Typical star image (after Gullstrand).

and horizontal is more marked for a wire of 0.81 seconds of arc diameter than for one of 0.94 seconds. The experimental results of Leibowitz point in the same direction. In Figure 1 he presents the combined acuity of vertical and horizontal in contrast to the obliques as affected by illuminance and pupil size. A large pupil and high illuminance give high acuities. In all cases the acuities for combined vertical and horizontal surpass that for the obliques and it will be noted that this superiority is in all cases greatest where the acuities are the highest and the gratings resolved the smallest.

Discussion

Is the cause of orientation preference to be found in the optical or in the sensory aspect of the eye? Does the accentuation of orientation preference in difficult observations throw any light on the problem? It is hard to visualize a retinal or postretinal mechanism explaining orientation preference, it is far harder to imagine one that will show little or no preference among stimuli recognized 95 percent of the time and a high preference among stimuli recognized only 20 percent of the time. It is believed, however, that a rather neglected aspect of ocular refraction gives an adequate optical explanation of orientation preference and would be more effective in the more difficult task. Discussion, however, requires a digression on the higher order aberrations of the eye.

The classical optical picture of a point image for a point source is not strictly true even for eyes of high acuity devoid of astigmatism or other refractive defect improved by glasses. The almost universal convention that stars have rays would not have arisen if our eyes had rendered the heavenly bodies as true points.

It was long ago shown by Gullstrand (1901, 1924) and reiterated by Lancaster (1943, 1952) and Boeder (1944) that light from each point of the object is not reassembled in points in the image but in caustic surfaces of complex form. As intercepted by the retina the refracted pencil from a single luminous point is typically an eight pointed star with a central brightest portion and less bright rays of which the vertical and horizontal are longer and more intense than the oblique. Such a refracted pencil as well as others less regular in form, it should again be emphasized, is compatible with a visual acuity above normal.

The variety of form shown by different eyes may readily be illustrated by looking at a star first with one eye and then with the

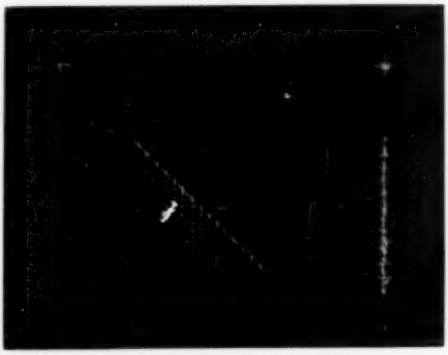

Fig. 4 (Weymouth). Diagram illustrating how a typical star image would combine to accentuate a vertical or horizontal object line as compared with an oblique object line.

other. The number and relative size of rays is often strikingly different. In the older writings this "star" effect, which is characteristic of the crystalline lens, since it is absent in aphakia, was ascribed to "irregular" astigmatism or to "irregularities" in the cornea or lens. Gullstrand was the first to show its true nature. Since it does not prevent excellent acuity, cannot be "corrected," and is only seen in some uncommon procedure such as stigmatoscopy, it is known to only a few workers and by them generally ignored.

To evaluate the effect of this aspect of refraction it will be helpful to review briefly the types of target with which orientation preference has been observed and then to compare the imagery of the star point with regular astigmatism. Orientation preference has been recorded with fine wires (60 times as long as wide), rectangles (four times as long as wide), grids of parallel lines, vernier acuity test objects, and the apparently unrelated but very interesting situation of halftones. Higgins and Stultz (1948) state, "Qualitative manifestations of this phenomenon can be seen quite easily by rotating a halftone print in a newspaper. If the area examined is of moderate uniform density, the halftone dots will appear as an array of lines when they are aligned either vertically or horizontally, but this systematic array will disappear when they pass through the visual field diagonally. The workers in the graphic arts are aware of this decreased visibility for diagonal lines." Letters have not been reported as showing orientation preference, although they are known to be affected by astigmatism.

That there must be some type of similarity between the star image point and astigmatism has apparently occurred to all workers on this problem. Comparison with regular astigmatism involves several points. How common is each type of "defect"? What effects have they in common? What differences?

Astigmatism of some degree combined or not with spherical errors is very common. In most cases low or moderate astigmatism in young persons may usually be corrected with good vision. It is much harder to state the incidence of the star image point. To identify what is being called the "star" image point require two conditions.

First the eye must be emmetropic either naturally or as far as can be effected by spheres and cylinders.

Second the image of a distant very small point of light must be examined subjectively, as in stigmatoscopy.

Under these conditions all "normal" eyes will show rays about the central bright point; these will vary widely in number and in length and brightness relative one to the other. The proportion which might be classified as eight rayed with the vertical and horizontal most prominent, or at least with conspicuous vertical and horizontal rays, is difficult to estimate.

No adequate stimgatoscopic surveys are on record; on the basis of students examined a guess of 10 to 20 percent might be hazarded. It should be noted that orientation preference in emmetropes with good vision has been proved but it has not been proved that all emmetropes show such preference. It will be recalled that in the five eyes tested by Ogilvie and Taylor there was a statistically significant difference in the degree of preference shown. Obviously an eye may fall in both categories, showing astigmatism and, when corrected, the star point. They represent aberrations of greatly differing order.

The two conditions have in common the phenomenon of orientation preference, with the preferred directions horizontal and vertical, since the axes of astigmatism and of the star rays are commonly so oriented. They differ in that the star shows a more marked preference the more difficult the task of recognition. This is not true of astigmatism.

Do these two types of refraction affect the total image? In astigmatism the image point becomes a line. If this line is parallel

to the edge of a letter the image of the edge remains sharp and clear, but if the focal line is perpendicular to the edge the contrast is greatly reduced and there is a gray transition zone between black and white. This is the well-known mechanism of the fan or clock-face astigmatic test chart.

For the star image the entire point is smaller even than in low-grade astigmatism and most of the light is concentrated in the central part. In consequence the image as a whole is good; as has been stated the acuity is often above normal. The intensity of the ray is so low that when they are perpendicular to the margin of the letter the reduction of contrast is slight. But if the image is that of a very fine wire so small that it is seen only part of the time the situation is quite different.

Assume a star point with the chief rays horizontal and vertical and the oblique rays not extending appreciably beyond the central disc. With a horizontal or vertical wire one ray would coincide with the main image and one would be directed to the side and so reduce the edge contrast of the image of the wire. With an oblique wire both horizontal and vertical rays would fall outside the image of the wire and thus all of the light of the rays would act to reduce contrast. Even if the amount of light in the rays is small, probably less than one tenth of the total, the difference between the two situations might well increase the number of correct answers for vertical or horizontal when correct answers form but a fraction of the total. For the data presented in the present paper it must be assumed that only the vertical ray extended significantly beyond the central disc. Such variations have been observed in stigmatoscopy.

In future work on this problem not only should the subjects be carefully refracted but they should also be examined by stigmatoscopy and the findings checked against the presence or absence of orientation preference.

Conclusions

We are justified in concluding that, in the reported cases, the orientation preference in linear stimuli of small angular size is an optical phenomenon. A certain proportion of men and women show an image "point" of small size consisting of a bright central area and eight rays of which the vertical and horizontal are usually the more prominent. These persons show good or unusually high acuity, but with very small linear stimuli the coincidence of image and ray direction leads to significantly lower thresholds in these directions. The phenomenon serves to emphasize a rather neglected aspect of image formation in the eye and to point up the importance of stigmatoscopy in understanding the finer points of ocular image formation.

950 West Jefferson Boulevard (7).

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MECHANICALLY INDUCED GLAUCOMA IN ANIMALS*

Preliminary perfusion and histologic studies

Milton Flocks, M.D., Isamu Tsukahara, M.D., and John Miller, M.D.
San Francisco, California

If prolonged increased intraocular pressure could be produced in animal eyes without directly interfering with the anterior chamber structures by the injection of occlusive materials, or other means, useful knowledge of the effect of glaucoma on the eye might be gained. If the elevated intraocular pressure could be produced in a controlled manner, the effect on the optic nerve, ciliary body, and retina could be studied in a controlled way. It has recently been suggested by one of us (M. F.[1]) that increased intraocular pressure injures the filtration angle also, even in those cases in which no peripheral anterior synechias are formed; and experimental means of studing this possibility would be invaluable.

Huggert[2] has recently reported a series of experiments in which he produced increased intraocular pressure in rabbits by obstructing aqueous outflow in one of three ways: (1) by cauterizing the aqueous veins, (2) by tying a ligature around the vessels at the posterior pole of the eye, and (3) by obstructing the filtration angle with suspensions of particles.

Other efforts for obstructing the outflow are well catalogued by Huggert[2] as follows:

"None of the three, in principle, different ways used here for blocking the outflow . . . is new. Similar experiments were made already as early as 1870 to 1890, first and foremost by Seber's school. Attempts with cauterization of the whole area around the limbus was made by, among others, Scholer and Heisrath, as well as painting the same area with silver nitrate by Heisrath and Bentzen. With the discovery of aqueous veins on the surface of the eye, came the possibility of cauterizing these vessels selectively. Such like experiments have been carried out by Weekers and Prijot. The effect of ligating one or more vortex veins was studied earlier by Scholer and Weber, and later on by Koster. Attempts at obstructing the outflow at the posterior pole have recenly been published by Bárány as well. Blocking of the filtration angle with different suspensions has already been done by Weber and Heisrath with, among other things, oils; and attempts to block it with bacteria are reported by Bentzen."

Increased intraocular pressure of one eye of 49 animals was produced by the application of a tight encircling rubber band around the equator of the eye, sewn to the sclera under the extraocular muscles somewhat in the manner of the encircling polyethylene tube that is sometimes used in the treatment of retinal detachment (fig. 1). Whereas the intraocular pressure usually readjusts itself quickly following the application of a too tight polyethylene tube, such readjustment does not readily occur when a rubber band is used because, as the eye gets smaller with the expulsion of aqueous, the rubber band also contracts and continues to apply external pressure.

Materials and Methods

1. Animals

The operation was performed on one eye of 46 rabbits, two monkeys, and one cat. The opposite eyes were left untouched as a control. The operation could be performed much more easily on rabbits because the rabbit eye is readily proptosed and these animals

* From the Division of Ophthalmology, Department of Surgery, Stanford University School of Medicine. This investigation was supported by Research Grant B-873(C3), United States Public Health Service. Presented in part before the Western Section, November 18, 1958, at San Francisco.

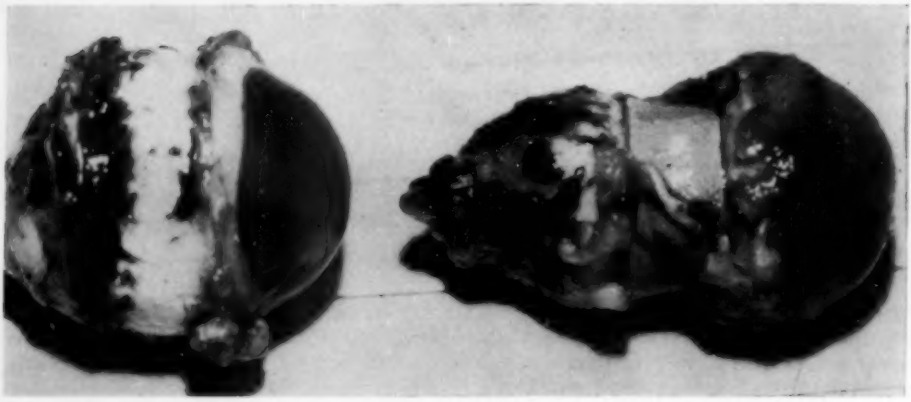

Fig. 1 (Flocks, Tsukahara, and Miller). Operated rabbit eyes of two rabbits after enucleation. The rubber band has been removed from the eye at the left so that it is ready for perfusion.

seemed to tolerate the operation much better than did the monkeys or the cat. Following the operation, marked edema of the lids of the monkeys and the cat developed but this did not usually occur in rabbits. General anesthesia was necessary to measure the tension of the monkeys and the cat but not of the rabbits.

2. The operation

Under general anesthesia the conjunctiva was circumferentially incised. Tenon's fascia was cut and the four recti muscles identified by passing a muscle hook beneath these tendons. A thin, flat, rubber band 4.0-mm. wide and approximately 0.75-mm. thick was cut so that it became a long rubber strip. A double-armed 4-0 black silk mattress suture was applied through one end of the cut rubber band. The other end of the rubber strip was passed beneath the rectus muscles until it encircled the globe and was lying flat around the equator. The rubber strip was stretched tight, compressing the globe firmly. The two needles of the suture were then passed through the opposite end of the rubber strip and tied re-establishing the circular nature of the rubber band. The excess rubber at the end of the strip was cut off. The suture was tacked to the sclera in four places. The conjunctiva was closed.

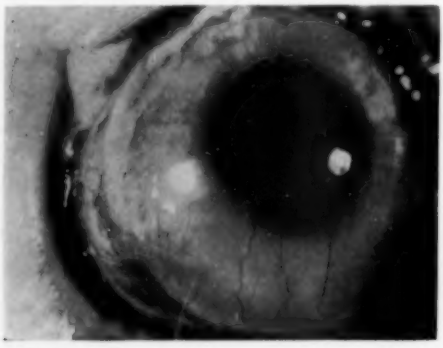

Fig. 2 (Flocks, Tsukahara, and Miller). Rabbit no. 30. The cornea shows marked vascularization at the periphery.

3. Ocular tension

At the conclusion of the operation the ocular tension measured with a Schiøtz tonometer was almost invariably between 70 and 100 mg. The tension of the rabbits' eyes was measured two or three times weekly under topical anesthesia. The tension of both eyes were always taken, the unoperated eye acting as a control. Tonography was performed on four of the animals at different stages in the postoperative course using a Mueller electronic tonometer and a Rectilinear Recorder (Texas Instruments, Incorporated).

4. Perfusion

At various times from two to 10 weeks after operation the animals were killed with intravenous nembutal and both eyes carefully removed. After enucleation the rubber band was immediately removed from the operated eye. Both eyes were then simultaneously perfused with saline and the facility of outflow determined. The perfusion apparatus was modified after Bárány.[3,4]

Each enucleated eye was supported in an inverted bell-shaped glass which contained saline moistened gauze. A No. 27 stainless steel needle was inserted through a small hole in the side of the glass and then into the anterior chamber of the enucleated eye. The needle was previously connected by way of polyethylene tubing to a 1.0 ml. pipet graduated in 0.01 ml. The pipet containing saline was mounted horizontally above the apparatus at an elevation giving the saline an effective constant hydrostatic pressure of 20 mm. Hg. The eyes were perfused within 15 minutes after enucleation for 90 minutes. The volume of outflow from each pipet was recorded at 10-minute intervals and plotted on graph paper. Excluding the first 10 minutes the facility of outflow was calculated from the average slope.

5. Histologic sections

After perfusion, the eyes were fixed in formalin, sectioned, and stained with hematoxylin and eosin.

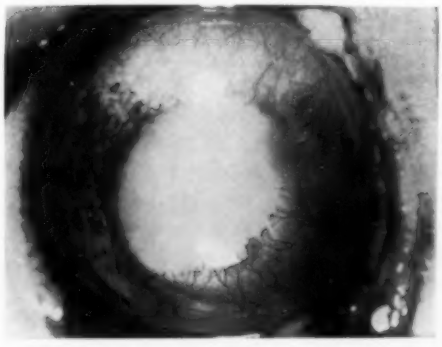

Fig. 3 (Flocks, Tsukahara, and Miller). Rabbit no. 15. A dense cataract is present. The periphery of the cornea is vascularized.

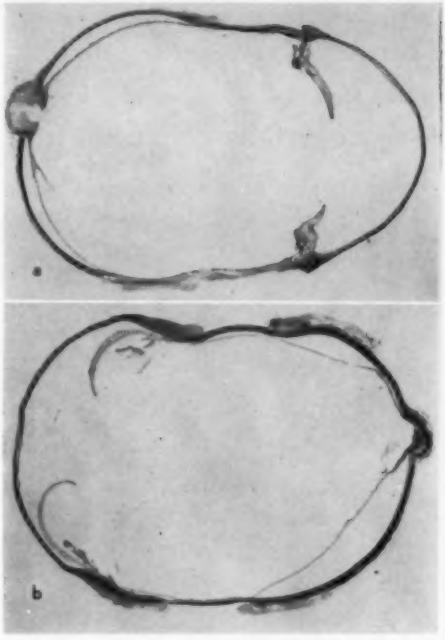

Fig. 4 (Flocks, Tsukahara, and Miller) (a) Enucleated 36 days postoperatively (Rabbit no. 16); and (b) enucleated 78 days postoperatively (Rabbit no. 30). Note the open angles and changes in the optic nerve. A mild granulomatous reaction on the outer surface of the sclera at the equator marks the site where the rubber band had been.

Results

1. Animals

The rabbits tolerated the operation well; the other animals not so well. Following the operation there was considerable edema of the lids of the monkey and the cat. General anesthesia was necessary to measure the tension in these animals, but not in the rabbits. In the monkeys and cats, difficulties in operative technique, infection, and other causes made the results in these animals unreliable and will not be referred to further in the rest of this paper. The following findings refer to the results in rabbits only.

TABLE 1

SUMMARY OF FACILITY OF OUTFLOW STUDIES

Rabbit No.	Perfusion C Values		Days Before Killed	Comment
	Normal	Operated		
1	0.416	0.0675	34	
2	—	—	25	
3	0.183	0.093	23	
4	0.186	—	25	Panophthalmitis
5	0.250	—	20	Panophthalmitis
6	—	—	23	Panophthalmitis
7	—	—	13	Panophthalmitis
8	0.204	—	15	
9	0.412	0.179	13	
10	0.271	0.233	14	
11	0.275	0.055	36	
12	—	—	4	Died on fourth postop. day
13	0.422	0.094	34	
14	0.237	0.097	35	
15	0.213	0.137	72	
16	0.53	0.125	36	
17	0.286	0.039	37	
18	0.275	0.105	21	
19	0.385	0.256	23	Vitreous leak in perfusion
20	—	—	34	
21	—	—	19	Panophthalmitis
22	—	—	16	Panophthalmitis
23	—	—	14	Panophthalmitis
24	—	—	14	Panophthalmitis
25	—	—	43	Panophthalmitis
26	—	—	13	Panophthalmitis
27	—	—	43	Phthisis bulbi
28	—	—	13	Phthisis bulbi
29	—	—	73	Phthisis bulbi
30	0.20	0.21	78	
31	0.15	0.116	74	Panophthalmitis
32	—	—	18	Panophthalmitis
33	—	—	15	
34	—	—	76	
35	—	—	37	
36	—	—	45	Panophthalmitis
37	—	—	15	Panophthalmitis
38	—	—	15	Died without perfusion
39	—	—	45	Died without perfusion
40	—	—	65	
41	0.19	0.10	58	
42	—	—	53	
43	—	—	58	
44	0.275	0.15	48	
45	—	—	52	
46	0.237	0.183	53	

2. Tension

The measurement of ocular tension in rabbits under local anesthesia, particularly in rabbits which have had eye operations, is by no means an extremely accurate procedure. The tensions were taken under local rather than general anesthesia to avoid frequent general anesthesia which could result in the death of some of the animals. The difference in intraocular pressure between the operated and unoperated eyes was readily detectable, however, and the course of the ocular tension could be followed fairly well. The tension in almost all of the animals declined steadily the first 48 hours to reach a level of 35 to 50 mm. Hg; and then decreased more slowly. Most of the animals still had elevated tensions two and three weeks after operation.

Tonography performed under general anesthesia on a few animals during the first 48 hours and at later intervals revealed a relatively flat tracing with very little aqueous outflow when compared to the control eye.

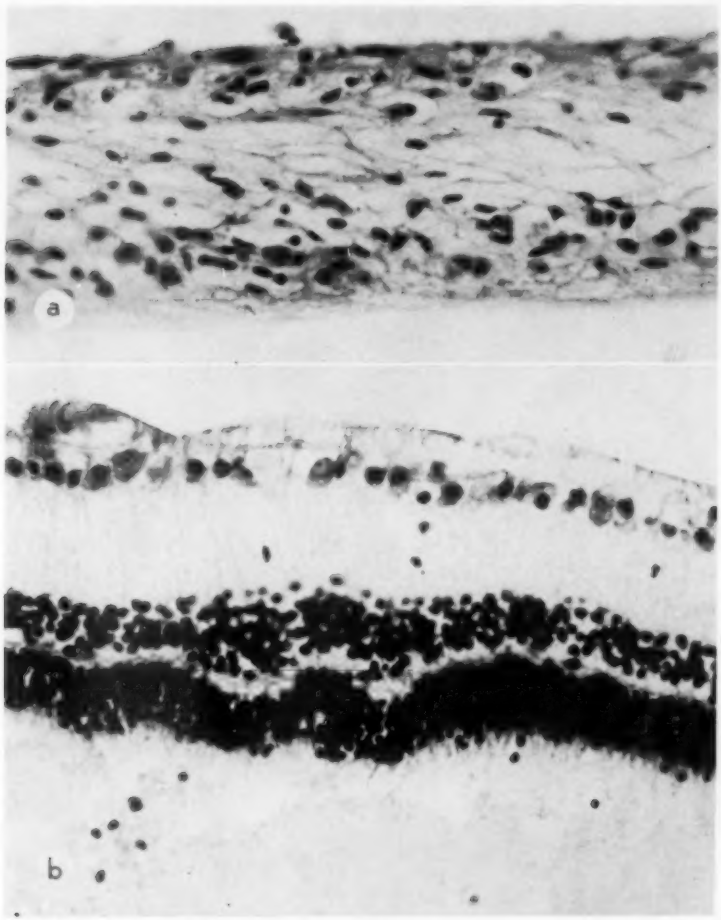

Fig. 5 (Flocks, Tsukahara, and Miller). (a) Rabbit No. 18. Marked pathologic changes in the retina. Compare with (b) normal rabbit retina.

3. Gross appearance

Mild to moderate conjunctivitis was common. In 14 eyes panophthalmitis developed. Two cases of phthisis bulbi resulted. In the remaining eyes the anterior chamber remained deep in all cases. The cornea began to show peripheral vascularization in all of the eyes beginning in the second week (fig. 2). The iris vessels in several of the eyes appeared dilated. In a few eyes a hyphema appeared which rapidly became absorbed. In one case a dense cataract developed (fig. 3). With the slitlamp frequently a faint beam and a few cells could be seen in the aqueous.

4. Perfusion

The facility of outflow in the operated eye after enucleation, with the rubber band removed, was definitely decreased in all but one case when compared to the fellow control eye (table 1). Except for the presence of increased intraocular pressure immediately after the operation, this was the most constant of all the findings in this study.

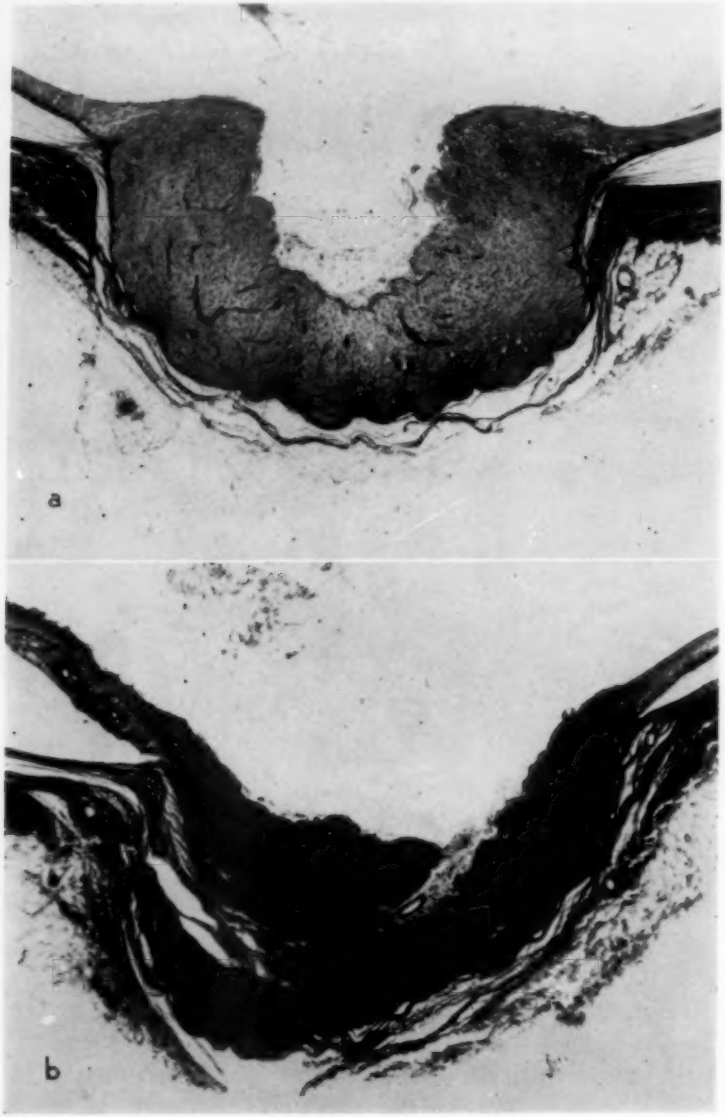

Fig. 6 (Flocks, Tsukahara, and Miller). (a) Rabbit No. 16. Optic nerve appears cupped. (b) Rabbit No. 30. Optic nerve early excavation.

5. Histologic findings

Most of the cases in which a frank panophthalmitis developed were not sectioned. The rest of the cases, the large majority, revealed little evidence of intraocular inflammation. In a few cases there were some acute and chronic inflammatory cells in the anterior chamber but the majority did not. A localized granulomatous reaction on the surface of the sclera invariably was present

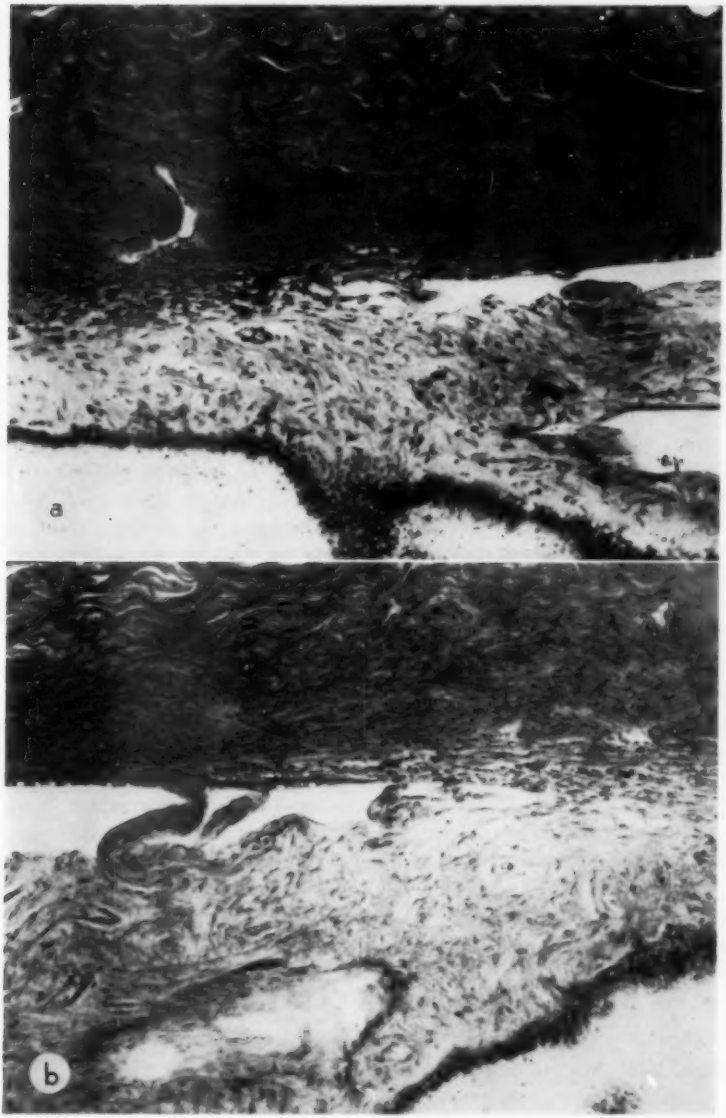

Fig. 7 (Flocks, Tsukahara, and Miller). Rabbit No. 30. (a) Filtration angle. No synechias are present. The meshwork appears slightly compressed but otherwise unremarkable. (b) The opposite angle of the same eye. Several iris pillars are seen. Synechias are not present.

revealing the site where the rubber band had been (fig. 4). Peripheral vascularization of the cornea was invariably seen. A constant and striking finding was marked degeneration of the retina usually involving all layers of the retina (fig. 5). The optic nerve usually showed signs of atrophy and in six cases was deeply cupped (fig. 6). The anterior

chamber was invariably deep. However, the histologic appearance of the rabbit filtration angle is so different than the human that we were unable to decide whether or not histologic evidence of damage to the angle was present (fig. 7). In only two cases were definite anterior synechias present.

Conclusions and Comment

There is no question that prolonged increased intraocular pressure in the rabbit eye can be produced by this method. However, the occurrence of inflammation and occasional intraocular hemorrhage are variables which make this method not the well-controlled way of producing glaucoma in animal eyes which would be desirable.

The constant finding of a decrease in facility of outflow in the enucleated operated eye with the rubber band removed is extremely interesting but, because of the many variables, no definite conclusions as to its cause can be drawn from this study. The absence of peripheral anterior synechias in virtually all of the cases rules out synechias as the chief cause of the decrease in the facility of outflow.

The striking pathologic changes in the retina throughout all of the layers of the retina even after only two weeks of elevated pressure suggest that the retinal damage is probably on a vascular basis and not related to the increased intraocular pressure alone.

It is reasonable to assume that the cupping of the disc and optic atrophy are largely due to the glaucoma. It is hoped that the technique can be improved so that a large series of monkey eyes can be studied; for in these animals pathologic changes in the filtration angle, which could account for the decrease in facility of outflow, could be recognized.

The relatively rapid decrease in intraocular pressure in the first 48 hours after operation in the presence of a flat tonographic tracing with virtually no outflow indicates that the fall in tension in this early period is due to a decrease in the rate of production of aqueous and is in general agreement with the findings of Huggert.[2] The reason for the immediate in vivo decrease in facility of outflow is not entirely clear. We are, at present, actively studying the rate of decrease of aqueous production in rabbits following the application of the encircling rubber band and the rate of increase in facility of outflow when the tension comes down to within nearly normal limits.

Summary

Increased intraocular pressure can be produced in rabbit eyes for periods of more than two weeks by the application of a tight encircling rubber band around the equator of the eye. After two weeks, if the operated eye is enucleated and the rubber band removed, perfusion studies reveal a decrease in facility of outflow, the cause of which is unknown. Histologic examination of these eyes reveals peripheral vascularization of the cornea, degeneration of the retina, and in some cases, cupping and atrophy of the optic nerve.

Clay and Webster Streets (15).

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CHANGES IN BACTERIAL DRUG SENSITIVITIES*

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The development of bacterial resistance to antibiotics is one of the major problems of modern medicine. This problem has been quietly creeping up on us for the last 15 years, commencing shortly after the introduction of the sulfa drugs and culminating in the epidemic of staphylococcus-resistant infections of the last couple of years.

This study started two years ago when several doctors were wondering about these resistances. Specifically, was there any seasonal variation of this resistance? Was the resistance still on the rise or had it leveled out at a plateau? Was there any difference between resistance of cultures taken on hospital patients and those taken in a private office? Lastly, which of the antibiotics was really the most efficient? We all had our favorities and there the discussion became quite heated.

Since there was an ideal source of data available, it was decided to attempt to analyze the records and see what some of the answers were. The records were obtained from the bacteriology laboratory of the New York Eye and Ear Infirmary and from a bacteriology laboratory conducted in the private office of one of the attending physicians. In both laboratories the drug resistance was tested by the standard Difco discs and results tabulated as resistant or sensitive. It was not attempted to grade the degree of sensitivity. During this study there was no change of personnel in either laboratory. Due to the special nature of the two laboratories only eye and ear, nose, and throat cultures are involved.

In this study, 1,827 cultures were divided into three groups (table 1). Staphylococcus was by far the largest participant with well over 1,000 cultures. It is interesting to note that there was a significantly higher percentage of these cultures in the hospital than in the private practice. Secondly, was the streptococcus, which was much more prevalent in the private practice environment. The third grouping was gram-negative rods. This includes Escheria coli, Proteus, Pyocyaneus, Bacillis subtilis, and numerous non-specific coliform bacilli.

In this study it was not attempted to separate the pathogenic from the nonpathogenic organism, nor to try to type the specific organism. It was felt that this would be compensated for with this large a sample. The standard error was determined and only those differences of over three sigma were felt to be significant. As representative antibiotics, aureomycin, Chloromycetin, penicillin, polymyxin, streptomycin, and terramycin were picked especially since they had been tested throughout the whole period.

Because of the necessity for a large number, the primary analyses were made only on the staphylococcus cultures. The first question that we shall consider is the question of

*Presented before the Southern Section, November 3, 1958, New Orleans.

TABLE 1

THE SOURCE AND DISTRIBUTION OF THE CULTURES IN THIS STUDY

	Streptotoccus	Gram-Negative Rods	Staphylococcus	Total
Private laboratory	288	170	387	845
Hospital laboratory	66	246	670	982
TOTAL	354	416	1,057	1,827

TABLE 2

STAPHYLOCOCCAL CULTURES SHOWN AS PERCENTAGE RESISTANCE TO THE ANTIBIOTICS DURING EACH QUARTER OF EACH YEAR

DRUG SENSITIVITIES

	Office						*Hospital*				
					Aureomycin						
	1954	*1955*	*1956*	*1957*	*Total*		*1954*	*1955*	*1956*	*1957*	*Total*
1st		0.0	12.5	15.2	9.0	1st	23.1	25.0	5.5	17.5	14.9
2nd		23.5	7.9	6.7	10.0	2nd	30.8	33.3	21.8	21.9	25.6
3rd		3.3	6.7		5.0	3rd	12.5	12.5	29.6		20.7
4th	21.4	2.8	26.4		17.9	4th	23.5	6.5	21.7		17.7
TOTAL	21.4	4.9	15.3	11.0	11.3	TOTAL	23.5	11.0	19.6	20.4	19.8
					Chloromycetin						
1st		2.6	25.0	23.9	16.0	1st	0.0	5.0	0.0	13.3	7.3
2nd		11.8	5.3	6.7	7.0	2nd	15.4	5.1	11.6	35.2	21.3
3rd		6.7	0.0		3.3	3rd	0.0	0.0	36.2		17.7
4th	17.9	11.1	30.2		21.4	4th	11.1	2.2	28.6		19.5
TOTAL	17.9	7.3	16.1	15.4	14.1	TOTAL	7.7	2.7	20.2	24.3	16.4
					Terramycin						
1st		18.4	18.8	41.4	29.0	1st	23.1	15.0	38.5	33.7	32.1
2nd		35.2	21.0	33.3	29.0	2nd	30.8	41.1	42.1	37.2	39.3
3rd		13.3	20.0		16.6	3rd	37.5	11.4	36.2		26.6
4th	17.9	19.4	45.1		32.5	4th	16.7	17.8	31.4		25.1
TOTAL	17.9	19.7	31.5	37.4	28.1	TOTAL	25.0	22.3	36.2	35.5	32.1
					Streptomycin						
1st		42.2	43.8	28.2	36.0	1st	7.7	16.7	26.4	21.3	21.4
2nd		64.6	15.8	8.9	21.0	2nd	46.2	15.4	20.3	21.9	21.6
3rd		0.0	10.0		55.0	3rd	28.5	25.0	17.0		21.3
4th	37.5	71.5	24.5		33.3	4th	17.6	19.6	19.0		19.0
TOTAL	37.5	42.1	21.2	18.7	26.3	TOTAL	26.0	19.6	20.4	21.7	20.9
					Penicillin						
1st		39.5	37.5	19.6	30.0	1st	23.1	29.4	9.1	54.6	35.0
2nd		52.9	26.0	8.9	23.0	2nd	77.9	15.4	24.6	35.1	30.7
3rd		26.7	26.7		26.7	3rd	37.5	17.1	48.9		34.4
4th	28.6	33.3	30.2		30.8	4th	27.8	13.0	83.0		58.2
TOTAL	28.6	36.4	29.2	14.3	27.8	TOTAL	40.4	16.8	48.0	44.4	39.6
					Polymyxin						
1st		94.8	68.8	76.1	82.0	1st	92.3	12.5	37.0	27.3	33.9
2nd		100.0	79.0	74.7	81.0	2nd	84.6	35.0	43.3	18.7	33.6
3rd		56.6	76.7		66.6	3rd	12.5	36.6	15.5		24.5
4th	89.9	66.6	81.1		79.2	4th	22.2	26.1	13.7		18.1
TOTAL	89.9	77.7	78.1	75.8	78.2	TOTAL	53.8	30.1	26.1	22.9	28.3

variation during the seasons. We divided the periods into quarters with the first three months being the first quarter and so on. In studying the data (table 2) it is seen that the third quarter is below the average in the majority of cases. Analytically the other three quarters has almost the same resistance rate, whereas the third quarter, July through September, is 1.75 sigma below that rate. This is not a significant figure, yet it is highly suggestive that possibly the bacteria also take a vacation in the summer and their antibiotic resistance is lower. This would bear further study at other centers.

The next question that might be considered is whether the resistance is still on the rise or has it levelled out. There are 18 categories in which this is seen. Six antibiotics, two locations, times three groups of bacteria. In almost every case they are fairly level throughout the three- or four-year period. The one glaring and interesting exception is Chloromycetin in the hospital. It was eight percent in 1954 and three percent in 1955. However, in late 1955 a group of eager second year residents started using this antibiotic a great deal. Consequently, in late 1956 there was a resistance of 33 percent, becom-

TABLE 3

STREPTOCOCCUS AND GRAM-NEGATIVE ROD CULTURES SHOWN AS PERCENTAGE RESISTANT FOR EACH ANTIBIOTIC FOR EACH YEAR

	Streptococcus								
	Hospital				*Office*				
	1955	*1956*	*1957*	*Total*	*1954*	*1955*	*1956*	*1957*	*Total*
Aureomycin	10.0	14.7	05.0	10.9	2.9	1.2	1.1	1.2	01.4
Chloromycetin	10.0	8.5	15.0	10.8	5.9	2.4	1.1	2.4	02.4
Penicillin	40.0	54.5	45.0	49.2	5.9	11.9	3.4	.00	05.2
Polymyxin	20.0	66.6	90.0	66.7	76.5	79.7	79.8	65.4	76.3
Streptomycin	10.0	28.5	5.0	18.4	52.9	47.6	49.4	59.3	52.0
TOTAL	40.0	44.1	45.0	43.7	5.9	1.2	4.5	3.7	03.5
	Gram-Negative Rod								
Aureomycin	39.3	44.6	16.4	34.5		32.4	36.5	45.1	36.3
Chloromycetin	29.8	36.7	22.2	30.7		21.6	38.0	25.8	28.5
Penicillin	73.2	81.3	75.0	77.4		83.8	95.2	96.7	89.9
Polymyxin	27.8	44.4	61.1	45.7		93.2	96.9	93.5	94.7
Streptomycin	8.9	34.8	27.7	26.7		35.2	19.0	29.0	27.9
TOTAL	35.7	44.8	36.1	40.0		41.9	96.8	93.5	72.0

ing 35 percent in one quarter in 1957. Could that be a cause and effect? Most studies on the large use of antibiotics would seem to indicate such.

Where do you expect that the higher resistance would be? In the hospital or in the office? Here the streptococcus (table 3) and staphylococcus can be taken together as the results are identical. With aureomycin, Chloromycetin, penicillin, and terramycin the resistance is higher in the hopsital than that in the office, usually significantly so. This to me was expected as the antibiotics were being used much more often in the hospital. However, polymyxin and streptomycin were much less effective in the cultures taken in the office. This I am at a loss to explain, since there was not a great use of them in the office. Against the gram-negative rods there was no real difference and none of the antibiotics were too effective (table 3).

Just how effective were the various antibiotics? Chloromycetin and aureomycin seem to tie for the honors here as the resistance rates are almost identical for both the staphylococcal and streptococcal infections. Against the rods streptomycin and Chloromycetin were most effective, but only in slightly over 70 percent of the cases. Aureomycin was a very close third.

In this study we have seen that in bacterial antibiotics the resistance was higher in the hospital. However, for two, it inexplicably was greatly higher in the office practice. It was further shown that of all the antibiotics tested Chloromycetin and aureomycin were the most effective over the full three-year period.

This possible summer decrease of resistance should be investigated further and if true, and the cause discovered, possibly this would aid us in the more effective use of antibiotics throughout the rest of the year.

Further, with the antibiotic resistance at an apparently steady level the withholding of certain antibiotics from general use is to be commended. Possibly this could be extended so that they would be more effective at a later date.

Marshall Taylor Doctors Building (7).

THE PATHOGENESIS OF SENILE MACULAR DEGENERATION*

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Senile macular degeneration affects 25 percent to 40 percent of our old age population according to our recent survey.[1] The greater percentage is among the older age groups—above 80 years of age. The degeneration varies in degree from minimal changes when central vision is reduced from 10 percent to 50 percent, to those with maximal involvement and complete loss of central vision.

Up to recently, there has been little opportunity to examine pathologically the eyes of old people with varying degrees of macular involvement, due to the inability to obtain eyes for such routine study. This opportunity arose at the Home for Aged and Infirm Hebrews of New York where a routine study of the eyes was instituted several years ago. These eyes were removed shortly after death and put in preservative and fixing solutions. They were then sectioned and stained with routine stains, usually hematoxylin and eosin, and occasionally with Mallory's trichrome stain. These sections were then studied for the changes in histologic structure normally accompanying old age.

The residents of the home were all examined clinically and periodically during their lifetime at the institution. Many of them had fundus photographs taken, especially if macula lesions were present. Many also were studied by the electroretinograph with special techniques using red and green filters to separate macular response from that of the rest of the retina. This work has already been reported by Jacobson, et al.[2] In this way considerable clinical and pathologic material was accumulated. This report deals with those pathologic specimens that showed changes in the macula, and specifically with those changes attributed to senile macular degeneration. An attempt will be made to build up the picture of senile macular degeneration from its earliest indications to the most advanced forms of the disease.

The 191 eyes that were obtained for study of the macula are classified in Table 1. Ninety-one eyes were relatively normal. Sixty-five eyes showed varying degrees of senile macular degeneration. Of the remainder, 14 eyes were myopic, with degenerative changes associated with high myopia. Nine eyes showed the degenerative changes related to diabetes mellitus. Four eyes had the Kuhnt-Junius type of degenerative change and eight eyes showed varying other types of degenerative changes, such as circinate retinopathy, glaucomatous macular disease, and changes attributed to central vein thrombosis. This report will be limited to the 65 eyes that had the senile macular degeneration uncomplicated by other types of retinal disease, as far as could be determined pathologically.

Forty-eight eyes had early macular de-

TABLE 1

CLASSIFICATION OF EYES FOR PATHOLOGIC STUDY OF MACULA

Type	Number	Percentage
Normal	91	47.6
Senile macular degeneration	65	34.0
Myopic	14	7.4
Diabetic	9	4.7
Kuhnt-Junius	4	2.1
Glaucoma	6	3.2
Circinate retinopathy	1	0.5
Central vein thrombosis branch	1	0.5
Total	191	100.0

* From the medical department of the Home for Aged and Infirm Hebrews of New York City. This study was supported by a grant from the U. S. Department of Health, Education, and Welfare, Institute of Neurological Diseases and Blindness (B-154). Presented in part at the XVII International Congress of Ophthalmology in Brussels, Belgium, September 1958. Presented before the Eastern Section, New York, November, 1958.

generative changes. These consisted of the following:

1. Diminution of the number of cone nuclei and processes.

2. Fragmentation of the external living membrane.

3. Changes in the pigment epithelium adjacent to the macula. These changes consisted chiefly of (a) loss of pigment granules, (b) migration of pigment cells, (c) proliferation of pigment cells.

4. Changes in the lamina vitrea, notably early drusen or colloid body formation.

5. Some evidence of mystic changes in the nerve fibre layer of Henle.

These changes can all be seen in the photomicrograph (fig. 1) of a section through the macula of the eye of a patient, J. F., a man, aged 91 years at death. There was evidence clinically of macular changes in the fundi, and the vision was reduced to 20/50 in each eye. There was also evidence of sclerosis of the retinal arteries and incipient cataracts. This section is fairly typical of most of the eyes showing early and minimal degenerative changes in the region of the macula.

As far as could be determined pathologically, there did not appear to be any changes in the choriocapillaris or choroidal vessels beyond the moderate to advanced sclerosis of choroidal arteries. No hemorrhages were noted, no inflammatory cells were present, and no closure of choroidal arteries. Similarly the retinal vessels showed no unusual changes beyond a moderate degree of thickening of the media.

The next group of eyes consisted of 10 specimens showing moderately advanced signs of macular degeneration. In addition to the above changes there were:

1. Some subretinal edema.

2. Greater proliferation and migration of the pigment epithelial cells into the subretinal space.

3. Greater loss of substance in the retina: (a) especially cone cells were fewer in number, (b) cystic changes in Henle's layer were more pronounced.

These changes can all be seen in Figure 2, photomicrographs of a section through the macula of the eye of a patient, S. H., aged 85 years at the time of death. The clinical examination of the fundi showed a moderately advanced macular degeneration in the left eye with vision reduced to 20/70. The right eye showed a circinate type of retinal involvement with advanced macular degeneration and vision reduced to hand movements. In this photomicrograph the left eye is seen showing the changes noted above.

Again it will be noted that the adjacent choriocapillaris and the choroidal vessels appear to be intact. There is no observable degree of thickening of the capillary walls. Similarly, one could see few changes in the retinal vessels close to the macula.

The last group of eyes consisted of seven specimens of advanced degeneration in the macula. These changes were:

1. Large cystic degeneration involving the entire macula.

2. Complete loss of the cone cells.

3. Hole in the macula.

4. Almost entire disappearance of the pigment layer on the choriocapillaris and the migration of most of the pigment cells into the subretinal space.

All of these changes were seen in varying degrees in this group of eyes. They are exemplified by the photomicrographs in Figure 3. This patient, B. C., aged 90 years at death, had bilateral macular degeneration seen clinically, with vision reduced to 20/200 in both eyes. Sclerosis of retinal arteries was also noted. This photograph shows all the changes noted above, except the presence of a hole in the macula.

Comments and Discussion

The pathologic changes in senile macular degeneration as exemplified in these three cases show a gradual but persistent progression of an atrophic process affecting the cone cells and the association fiber cells in the region of the fovea centralis and surrounding macular area. A reactive process in the

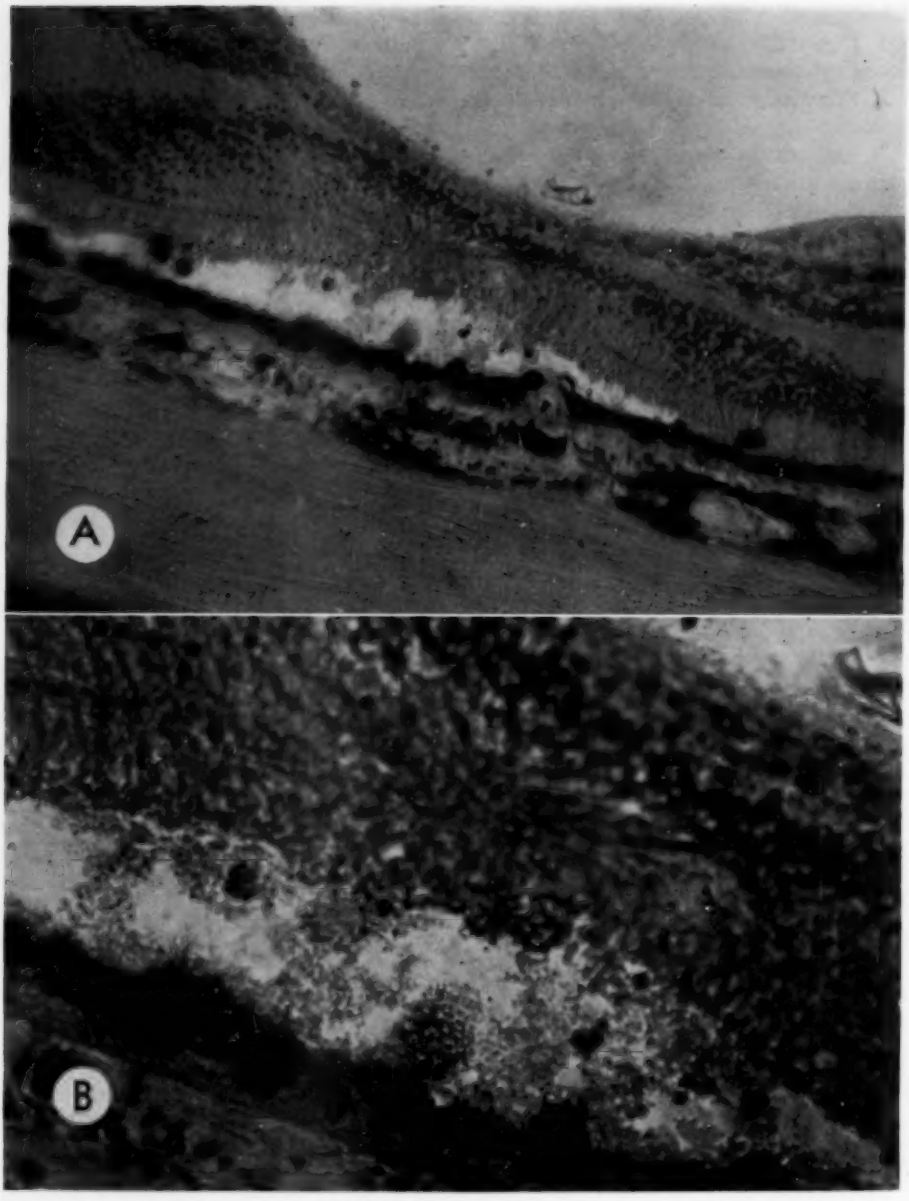

Fig. 1 (Kornzweig, Feldstein, and Schneider). (A) Low-power view of macula, showing early changes of senile macular degeneration. (B) High-power view of foveal area.

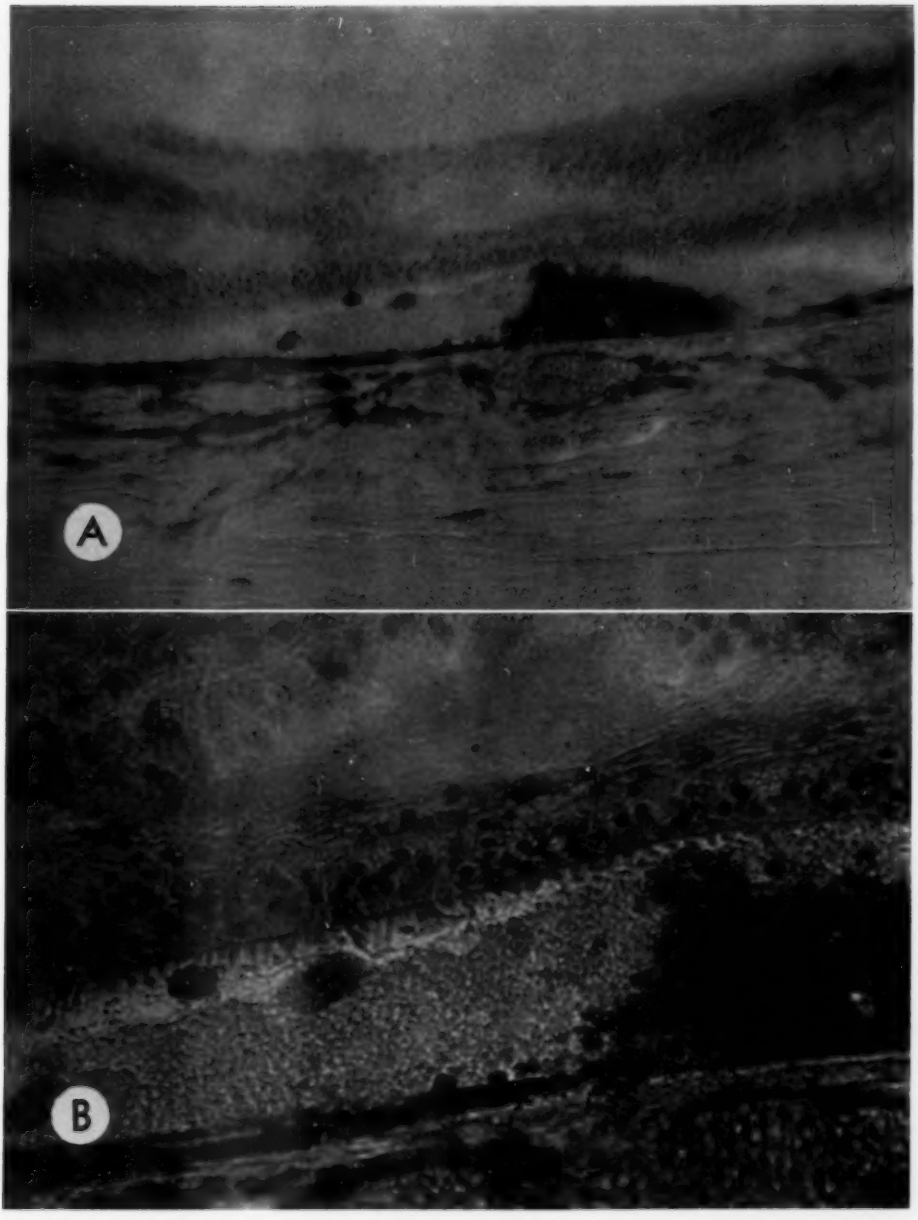

Fig. 2 (Kornzweig, Feldstein, and Schneider). Low-power view of macular area, showing moderately advanced senile macula degeneration. (B) High-power view of foveal area.

adjacent pigment layer results in loss of pigment granules, proliferation, and migration of pigment cells. Cystic spaces in the fovea and nerve-fiber layer also indicate areas of atrophy of cell and fiber substance and, possibly, also areas of exudation, the contents having been dissolved out or lost in the process of fixation, embedding, and staining of the sections.

There is no evidence of inflammation such as round cells or polymorphonuclear cells. There is very little, if any, glial proliferation. There are no visible hemorrhages or the late effects of such hemorrhages—such as scar tissue or granular debris. The lamina vitrea is intact and the choriocapillaris appears to be unaffected. Similarly, the retina adjacent to the macula also appears to be intact. It appears as if this portion of the retina acts as a separate organ subject to influences that do not affect the other parts of the retina.

Most recent writers on the subject, Behr,[3] Elwyn,[4] Klien,[5] and Falls,[6] have expressed the view that senile macular degeneration, originally described by Haab,[15] belongs to the heredo-degenerative group of diseases. This presupposes a genetic predisposition to aging and death at a given period in life of a specific tissue apart from the physical conditions existing in other parts of the body of the same individual. This death of tissue is described as an abiotrophy according to Gower, and is explained as an accumulation of metabolites in a given cell which the cell is unable to discard. This eventually destroys the ability of the cell to function properly and it then atrophies and dies. It has also been expressed as a failure of the enzymatic systems in the cell which are genetically predetermined. The time of the occurrence of the failure is usually associated with the periods of greater stress in the individual, such as secondary dentition, adolescence, maturity, and old age.

Other authors, Parsons,[7] Friedenwald,[8] Rones,[9] Wood,[10] Duke-Elder,[11] have attributed the cause of this disease to sclerosis of the choroidal vessels in the region of the macula and especially of the choriocapillaris. There is thickening and hyalinization of the choriocapillaris with obliteration of many vascular channels. This eventually leads to loss of nutrition to the adjacent macula-foveal area and gradual degeneration and loss of function. The sclerotic changes in the choroid may be widespread, but the limitation of the retinal changes to the macula indicates the greater vulnerability of this region.

The eyes that have been examined in this study do not bear out this conclusion. The choriocapillaris and choroidal vessels appear to be fairly normal under the microscope. There is no difference in the blood vessels in this area than in other parts of the choroid, and what difference there is appears to be in favor of a more abundant circulation here than elsewhere. The pathologic reports of cases of senile macula degeneration—of which there are but few (Nagel,[12] Harms,[13] Behr,[14] Klien[5])—all showed a fairly normal choriocapillaris and choroid, and an intact lamina vitrea.

Another possible cause of senile macula degeneration must also be considered. The circulation in the retina may be a much more important factor than it was believed to be heretofore. The macula-foveal area, because of its active metabolism and great need for oxygen, depends not only on the choroidal circulation but also on the retinal circulation. This area of the retina seems to act differently than any other part. It is more sensitive to toxins and injuries. Hypertensive, nephritic, and diabetic changes in the retina are more pronounced in the macula area. The viability and good function of the macula may persist even when it is separated from the choroid for short periods of time as in central serous retinopathy and in retinal detachment. This would appear to indicate the importance of the retinal circulation for the normal functioning of the macula. Gradual obliteration by a sclerosing process in the retinal arterioles in this region may cut down the nutrition and oxygen supply sufficiently

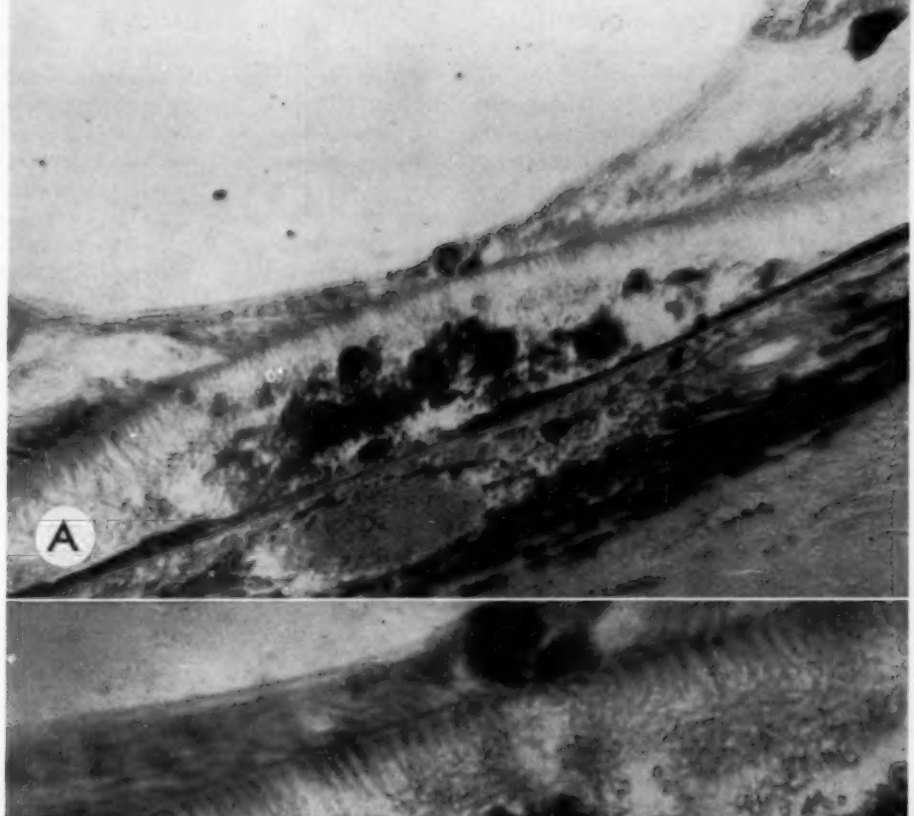

Fig. 3 (Kornzweig, Feldstein, and Schneider). (A) Low-power view of macular area, showing advanced macular degeneration. (B) High-power view of foveal area.

to produce the gradual progressive changes noted above in senile macula degeneration.

The changes in the pigment epithelium and the presence of serous exudates can be accounted for by the known phagocytic activity of the pigment cells. The degenerative process in the outer retinal layers—specifically the cone processes—causes a reaction in the pigment layer which stimulates it to proliferate and migrate to the degenerated cells in an effort to remove the debris.

The presence of senile macula degeneration in 25 to 40 percent of the aging population would seem to point to a more general etiology, one associated with the aging process itself. Sclerosis of the arterial circulation bears such a relationship. Further studies of the retinal circulation as it relates to the macula are being undertaken in an attempt to clarify this subject.

Summary and Conclusion

The pathogenesis of senile macula degeneration has been presented from a study of 65 eyes in elderly residents of the Home for Aged and Infirm Hebrews of New York. These changes consist chiefly of a loss of substance in the fovea and adjacent macular area, and a reactive change in the neighboring pigment layer and lamina vitrea. Several theories are mentioned that help to explain the sequence of events, with the major emphasis on the thought that this condition belongs to the heredo-degenerative diseases. The hypothesis is also presented that this area of the retina acts like a peripheral vascular end organ. Its gradual deterioration may be due to a slow obliteration of the blood supply and consequent anoxia and loss of nutrition, partly from the adjacent choriocapillaris and partly from the retinal circulation. The retinal circulation may be of greater importance in this condition than it was thought to be heretofore.

1 East 63rd Street (21).

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CHARACTERIZATION OF THE INTRACELLULAR PROTEOLYTIC ENZYME IN THE LENS*

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In 1957 Zeller and Devi,[1-3] using a sensitive manometric method, reported the presence of an intracellular proteolytic enzyme in the various parts of the rabbit eye. They also found highest esterase activity in the lens and very feeble activity in the cornea. In this paper the results of an investigation on the characterization of this intracellular enzyme are reported. It will be seen from the results presented in this paper that the intracellular enzyme as occurs in the lens is very similar in nature to cathepsin C.

The method of estimation of this enzyme was based on the liberation of carbon dioxide in a bicarbonate medium by the acid formed from the hydrolysis of synthetic esters by the enzyme and can be represented by the following equation: $C_6H_5CH_2CHNH_2COOC_2H_5 + E \rightarrow C_6H_5CH_2CHNH_2COOH + C_2H_5OH$. The conventional manometric method was used to measure the carbon dioxide thus liberated from the reaction mixture.[4,5]

The reaction velocity, a measure of the enzymic activity, is expressed here in terms of μ-liter of CO_2 liberated per hour per gm. of the wet tissue. Substrate blanks and enzyme blanks were separately carried out in the complete system without enzyme or substrate in the reaction mixture.

MATERIALS

Cattle eyes were removed immediately after killing of the animal in the slaughter house and transported in packed ice to the laboratory. Homogenates of the lens and other parts of the eye were prepared in normal saline in a Virtis homogenizer within a very short period of time. The temperature was maintained at O°C. as closely as possible throughout the process of dissection and homogenization. The solutions of the substrate and inhibitors were freshly prepared just before the experiment.

Substrates. Phenylalanine ethyl ester (PEE), tyrosine ethyl (TEE), benzoyl tyrosine ethyl ester (BTEE), lysine ethyl ester (LEE), glycine ethyl ester (GEE) were purchased from Sigma and Company. Benzoyl arginine ethyl ester (BAEE), benzoyl histidine ethyl ester (BHEE), and carbonaphthoxyphenylalanine were kindly supplied by Dr. E. A. Zeller of Northwestern University, Chicago.

Enzymes. Chymotrypsin was purchased from Armour and Company, Chicago.

Inhibitors. Ovomucoid, soybean trypsin inhibitor were purchased from Worthington Corporation, New Jersey; diisopropylfluorophosphate, cetylpyridinium chloride, and p-chloro mercuribenzoate were obtained from Fischer Scientific Company, New York.

RESULTS AND DISCUSSION

Table 1 shows the experimental conditions employed in the measurement of the esterase activity of the lens homogenate from the rate of hydrolysis of PEE in a bicarbonate medium. As can be seen in Table 2, the highest activity was found in the lens. Next to this in activity was the vitreous body, which represented approximately 50 percent of the lens activity. The retina exhibited only seven or eight percent, whereas cornea was prac-

TABLE 1

CONDITIONS IN THE MEASUREMENT OF THE ESTERASE ACTIVITY OF LENS HOMOGENATE

1.0 ml. lens homogenate
0.7 ml. $NaHCO_3$ (0.18M)
0.3 ml. distilled water
1.0 ml. 0.075 M PEE

* From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University. Presented before the Eastern Section, New York, November 22, 1958.

TABLE 2

DEGRADATION OF PHENYLALANINE ETHYL ESTER BY THE VARIOUS PARTS OF THE STEER EYE

No. of Investigations	Parts of the Eye	Q CO_2 in μ-liters/hr/gm of enzyme
10	Lens	850 ± 80
10	Vitreous Body	408 ± 60
*	Retina	60
*	Parts of Lens Capsule	210
*	Cornea	0

* Only six separate investigations were made with six eyes.

tically free from esterase activity. The absence of esterace activity in cornea might be due to the difficulty of preparing a homogenate of it as cornea is a very tough material and resists homogenization. This point needs further investigation.

As the main object of this communication is to characterize the lens enzyme, and once it is known that the highest enzymic activity is found in lens, only lens homogenate was subsequently used in all experiments unless otherwise mentioned.

Table 3 shows the liberation of CO_2 expressed in μ-liters per hour by one gm. of the wet tissue in a reaction mixture of total volume of 3.0 ml. containing different substrates. Although lens enzyme is capable of catalyzing the hydrolysis of tyrosine ethyl ester (TEE),[6] phenylalanine ethyl ester and very slightly benzoyl arginine ethyl ester, it will be seen that the best and ideal substrate for this study is phenylalanine ethyl ester (PEE).[7] The hydrolysis of PEE by lens enzyme as can be seen in Figure 1 is proportional to the time of incubation up to 15 to 20 minutes; in other words, follows the zero order reaction, then attains the first order kinetics. This is because the initial substrate concentration was high and practically all of the enzyme combined with it and remained so for a considerable part of the reaction, but later on the substrate concentration dropped and the course of the reaction followed first order kineics. The possibility of product inhibition was excluded by the fact that the addition of further substrate at this point (after 15 minutes) restored the original reaction velocity. The velocity of reaction also shows a linear increase with the increase of enzyme concentration (fig. 2). It should be recalled that PEE has also been shown to be the best and the ideal substrate for chymotrypsin and intracellular protease. This sug-

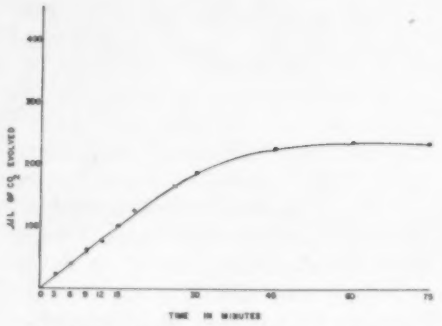

Fig. 1 (Devi). Degradation of phenylalanine ethyl ester by lens homogenate.

TABLE 3

DEGRADATION OF VARIOUS SYNTHETIC AMINO ACID ESTERS BY STEER LENS HOMOGENATE

Substrate Used	Percent Degradation
Phenylalanine ethyl ester (PEE)	80–90
Tyrosine ethyl ester (TEE)	80–90
Benzoyl tyrosine ethyl ester (BTEE)	80–90
Benzoyl arginine ethyl ester (BAEE)	20
Benzoyl histidine ethyl ester (BHEE)	nil
Lysine ethyl ester (LEE)	nil
Glycine ethyl ester (GEE)	nil
Carbonaphthoxyphenylalanine	10

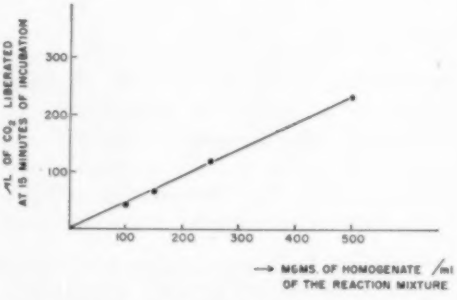

Fig. 2. (Devi). Effect of enzyme concentration on the activity of the steer lens homogenate.

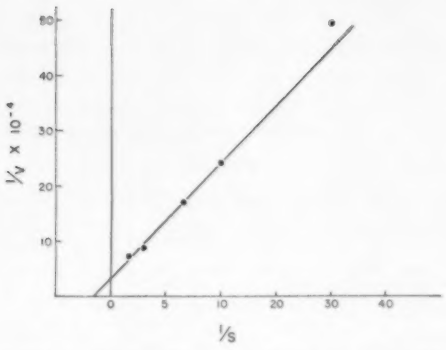

Fig. 3 (Devi). Effect of substrate concentration (L. B. method) on the degradation of PEE by steer lens homogenate.

gests that the lens enzyme is identical with chymotrypsin.

Both enzymes have the same pH optima (6.8) in the hydrolysis of PEE and both are stable when standing at room temperature for 72 hours.

The Michaelis Menton constant (Km) was determined at pH 6.8 after first ascertaining that the rate of reaction at this pH was proportional to time, until 80 percent of the substrate had been cleaved. The reaction velocity under the conditions of the experiment was a function of the substrate concentration. Figure 3 shows the data thus obtained. Applying the conventional Lineweaver- Bark equation

$$\frac{1}{v} = \frac{Km}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

where Km is Michaelis and Menton constant, v is over-all velocity of the enzymatic reaction, V_{max} is the maximum velocity, and [S] is the substrate concentration, the Km value (calculated by the extrapolation of the linear portion of the plot) is 3.0×10^3 which is of the same order as that obtained for chymotrypsin.

Table 4 shows the effects of various inhibitors of proteolytic enzymes on the esterase activity of the lens homogenate. The maximum inhibition of the esterase activity was found with diisopropylfluorophosphate. With soybean trypsin inhibitor and cetylpyridinium chloride, only 40 to 50 percent of the esterase activity was suppressed. Ovomucoid blocks only 28 percent of the activity, while p-chloromercuribenzoate blocks only 24 percent. The same inhibitors, at the same concentration, inhibit to the same extent the esterase activity of crystalline chymotrypsin. The concentration of chymotrypsin used throughout these studies was adjusted to give esterase comparable to the lens activity.

As can be seen from Table 4, both enzymes under the same experimental conditions suffer the same degree of inhibition of esterase activity. These results clearly indicate the similarity of the lens enzyme with chymotrypsin.

The question arises whether this evolution of CO_2 by the lens in the reaction media is due to the high content of lactic acid in the lens. As mentioned before, the enzyme blank in each experiment is substracted from the main reaction before the Q values are calculated.

TABLE 4

INHIBITION OF ESTERASE ACTIVITY OF THE LENS HOMOGENATE BY DIFFERENT INHIBITORS OF PROTEOLYTIC ENZYME

Inhibitors	Chymotrypsin		Lens	
	Q CO_2 in μL/gm./hr. × 10^6	% of Inhibition	Q CO_2 in μL/gm./hr.	% of Inhibition
	45.2		580	
Ovomucoid	32.6	26	420	28
Soybean trypsin inhibitor	26.7	41	300	48
Diisopropyl-fluorophosphate	16	65	128	79.3
Cetylpyridinium chloride	29	38	261	44.5
p-Chloromercuribenzoate	35	25	380	24

It seems obvious, especially from the study of the stability, specificity of these two enzymes, similarity of Km values, pH optima, and the degree of inhibition of their esterase activity by different inhibitors, that the lens enzyme is in all probability similar in nature to chymotrypsin.

The primary function of an extracellular protease (for example, trypsin and chymotrypsin) is to hydrolyze protein. However, the function of the lens enzyme is not clear. While it is similar in substrate specificity to that of chymotrypsin, it does not appear to function as an extracellular protease owing to the high concentration of protein in the lens. It is suggested, therefore, that its function may be one of synthesis rather than degradation since intracellular proteases (for example, cathepsin C) have been shown to synthesize higher peptides by transpeptidation. Perhaps this lens enzyme at this high concentration is required for maintaining the physical and chemical state of the protein in such a way that lens transparency is secured at all times of the lens' life.

Summary

1. An intracellular proteolytic enzyme has been detected in the steer lens which is very similar to cathepsin C.

2. The enzyme is highly inhibited by diisopropylfluorophosphate, ovomucoid, and a less degree of inhibition was found in the case of cetylpyridinium chloride, soybean trypsin inhibitor, and p-chloromercuribenzoate.

630 West 168th Street (32).

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OSMOTIC PRESSURE MEASUREMENTS OF INTRAOCULAR FLUIDS BY AN IMPROVED CRYOSCOPIC METHOD*

Physiologic significance relative to aqueous humor dynamics

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It has been generally assumed that the bulk flow of aqueous humor, from the posterior chamber through the pupillary space into the anterior chamber and out at the angle, is maintained by an osmotic pump mechanism activated by an osmotic pressure gradient increasing from the plasma to the posterior chamber fluid. In experimental support of this concept are the many osmotic pressure measurements of the aqueous humor and plasma made in the rabbit, man, and other animal species, recently reviewed by Levene.[1] Because of the relative ease of removing aqueous humor from the anterior chamber in sufficient amounts for analysis,

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measurements have been made almost exclusively upon this fluid.

The observed hypertonicity of the aqueous humor of the anterior chamber to the plasma as well as the existence of a unidirectional flow of fluid from the plasma into the posterior chamber of the rabbit eye has led to the assumption that the aqueous humor of the posterior chamber is also hypertonic to the plasma.

The only measurement of osmotic pressure of the aqueous humor of the posterior chamber was made by Kinsey[2] in 1951 (vapor pressure method of Baldes[15]), who reported that the osmotic pressures of the aqueous humors of the posterior and anterior chambers of the rabbit eye were equal and that both were higher than that of the plasma, by an amount equivalent to 3.0 mM/kg. H_2O of NaCl. This relationship was adduced as evidence in support of the secretion of aqueous humor arising in the posterior chamber of the rabbit eye. Moreover, in view of the observed isotonicity of the aqueous humors of both chambers, Kinsey concluded that no net exchange of water occurred between the plasma and the aqueous humor of the anterior chamber. Consequently, the rate of flow of aqueous humor out at the angle could be considered to be essentially identical to the rate of formation of aqueous humor in the posterior chamber and to the rate of flow through the pupillary space into the anterior chamber.

The concept of the fundamental dependence of aqueous humor formation upon the hypertonicity of the aqueous humor in the posterior chamber to the plasma has served as a basis for the explanation of the suppression of aqueous humor formation elicited by the intravenous administration of Diamox. Urrets-Zavalia and Remedi[3] found a fall in the electric conductance of the anterior aqueous humor of the dog, of the order of 5.0 mM/liter equivalent of NaCl, after an intravenous injection of Diamox. While the use of electric conductance as a basis for the estimation of relative osmotic pressure is open to considerable question, the significance of these results is also doubtful in view of the absence of simultaneous corresponding plasma measurements.

Langham and Lee[4] concluded that intraocular dynamics were not essentially affected by Diamox but at the same time suggested that the temporary decrease in rate of flow of the aqueous humor after Diamox may have resulted from disturbance of the normal osmotic balance between the aqueous humor and blood, although direct measurements of this relationship were not made. Similarly, Davson and Luck[5] suggested that the action of Diamox upon the dynamics and chemistry of the aqueous humor was primarily a reflection of a presumed decrease in the osmolarity of the aqueous humor in relation to the plasma. On the other hand, Levene[1*] and Tanner and Harris,[6] using the Fiske osmometer, found that the intravenous administration of Diamox did not significantly alter the osmolal relationship between the aqueous humor of the anterior chamber and the plasma.

While this paper was in preparation Auricchio and Wistrand,[7] using the Hill-Baldes thermoelectric vapor pressure method, reported the failure of intravenous Diamox to alter the tonicity of the aqueous humors of the rabbit after 65 to 75 minutes. Direct comparison of the posterior and anterior chamber aqueous humors could not be made since the posterior and anterior chamber fluids were removed from eyes of different rabbits and the types of anesthesia employed prior to tapping the posterior chamber and anterior chamber aqueous humors were not the same (local and general, respectively).

Because of the particular significance attributed to the maintenance of an osmotic pressure gradient between the plasma and the aqueous humor relative to the dynamics of aqueous humor formation, and to the mode

* We are grateful to Dr. Levene for making available to us a preprint of his paper. We would also like to express our appreciation to Dr. Kinsey for bringing to our attention the unpublished work of Dr. Levene.

of action of Diamox upon the eye, this investigation was undertaken to ascertain the osmotic pressure relationship between the aqueous humors of the anterior and posterior chambers of the rabbit eye and the plasma, and to determine the effect of Diamox upon these relationships.

The pursuit of this study was facilitated by the availability of a sensitive, accurate, and relatively simple microcryoscopic method.

The results of this study show that in normal untreated rabbits the osmotic pressure of the anterior chamber aqueous was significantly greater than that of the posterior chamber aqueous and of the plasma. On the other hand, no osmotic gradient was found to exist between the aqueous of the posterior chamber and the plasma. The results further indicate that after an intravenous injection of Diamox, the osmotic pressure of the anterior chamber aqueous was decreased to a significant degree while that of the posterior chamber aqueous and the plasma were not substantially affected by the drug.

Methods and Procedures

Freezing-point determination

The freezing-point apparatus and method of Ramsay and Brown[8] were used with slight modification. Because of the high degree of sensitivity and reproducibility as well as its general applicability for handling very small volumes of fluid, the apparatus and method are described in detail.

*Description of instrument**—The instrument is divided essentially into two sections (fig. 1): the control panel and light source on the right and the freezing bath on the left. The bath (fig. 2) in turn is divided into four subcompartments: the impeller, the specimen holder and thermometer, the microscope, and the cooling section with the heating element set between the impeller and cooling unit.

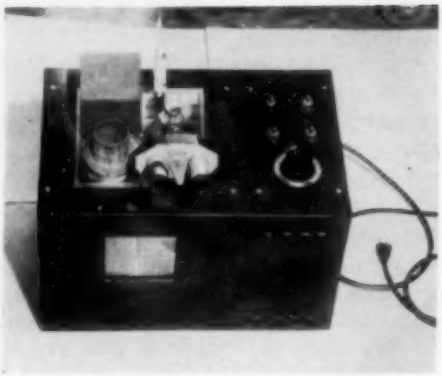

Fig. 1 (Kass and Green). The Ramsay-Brown freezing-point depression instrument, top view. Control panel on right. Bath compartment on left.

In order to minimize heat exchange paths and condensation surfaces, leucite plastic was used wherever feasible for the construction of parts within the bath.

Operation of instrument—With the bath level kept at a predetermined set height, the bath (30 percent methanol) was cooled by adding dry-ice and absolute methanol to the Pyrex outer vessel of the cooling unit. Efficient circulation was maintained continuously by means of the impeller so that within 20 to 30 minutes the bath attained the required temperature of several degrees below zero. The inner cooling vessel containing a dry-ice-alcohol mixture was immersed into the alcohol of the outer vessel. The temperature was raised slowly by adjusting the depth of immersion of the inner vessel by means of a movable collar. Simultaneous manipulation of the heating system, via a dial on the control panel, regulated the bath temperature so that during the thawing of a frozen specimen a temperature change of 0.01°C. per minute or any other desired optimum could readily be maintained.

Minor modifications made on the Ramsay-Brown instrument—A mercury-in-glass thermometer (Heidenhain), 15.0 cm. in exposed length, was used which had a working

* The instrument was constructed for us by C. N. Wood Manufacturing Company, 6208 Germantown Avenue, Philadelphia 44, Pennsylvania.

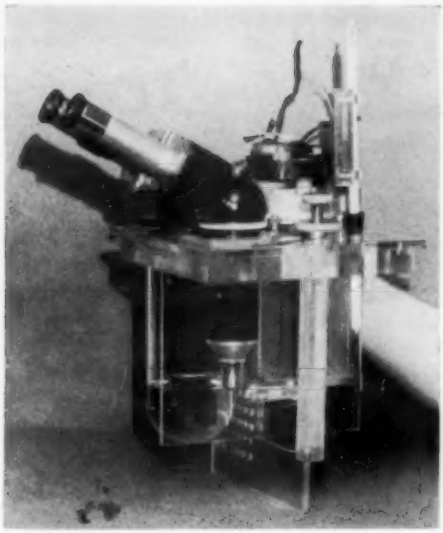

Fig. 2 (Kass and Green). The bath compartment removed from the Ramsay-Brown instrument showing contents of its four subcompartments: Microscope, inner and outer cooling vessels, heater and impeller, thermometer and specimen holder.

temperature range of from O to −1.0°C. and was calibrated in 0.01°C. Readings were estimated to the nearest 0.005°C. A magnifying bar of leucite, clamped to the thermometer, contained a reference crosshair which was set at a given thermometer reading. To reduce possible errors due to parallax a chin rest for the observer was stationed in front of the instrument and thus a constant eye-thermometer relationship was maintained. The heating unit used a rheostat arrangement so that small changes in resistance could be effected with the turn of a dial instead of a fixed-point switch system. Eye strain was decreased with the use of a binocular microscope head and a ×10 magnification.

Advantages of the Ramsay-Brown method—The apparatus was designed to determine the freezing-point of small volumes of aqueous solutions and avoids the difficulties of undercooling by first freezing the sample and then determining the thawing point. It offers several distinct advantages over others employed for this purpose:

It is relatively simple with regard to construction, operation, and maintenance.

The method works best with volumes of the order of 10^{-3} to 10^{-4} mm.[3] Thus the freezing-point of aqueous humor from single eyes is readily determined without the necessity of pooling samples from several eyes. The disadvantages attendant upon pooling of samples, such as evaporation, loss of CO_2, dissolution of aqueous or plasma constituents when standing under a protective fluid at room temperature for long periods, excitement incident to a group of animals brought into the laboratory at one time, and so forth, are no longer possible sources of error. This ability to measure individual rather than pooled samples is obviously highly desirable.

The accuracy of the comparative determinations is greatly enhanced by the fact that samples of blood plasma and posterior and anterior chamber aqueous of a given rabbit can be measured simultaneously in the same system under one set of bath and temperature conditions. Any errors incurred are thus identical for all three samples and would not affect their relationship with each other.

Obtaining the specimen

An adult pigmented rabbit weighing between four and six pounds was brought into the laboratory and confined in a wooden box one hour prior to the experiment. The rabbit's right eye, previously treated with one drop of 0.5-percent pontocaine hydrochloride solution, was proptosed, flushed with isotonic saline and blotted dry, and then tapped to obtain the posterior chamber aqueous humor and the anterior chamber aqueous humor in that order. The posterior chamber aqueous humor was removed by means of a capillary micropipette with a sealed-in platinum 26-gauge needle as previously described.[9] The fluid in the pipette was immediately expelled with the aid of syringe and plunger under a layer of silicone oil contained in a watch glass and the latter was

covered with a Petri glass cover.* The aqueous humor from the anterior chamber was removed as previously described[10] and expelled as above.

Blood plasma was prepared from the whole blood withdrawn from the central ear artery into a Siliclad-treated† tuberculin syringe containing a layer of paraffin oil. The blood was transferred‡ under oil, to a 5.0 ml. centrifuge tube and spun in a refrigerated centrifuge (International PR-1) at 1,000 rpm for 10 minutes. The plasma was then withdrawn under oil into a tuberculin syringe and delivered to a watch glass containing paraffin oil.§

After paracentesis of the right eye and the withdrawal of a blood sample, the left eye was tapped as described above, either immediately in the normal untreated rabbits or at some specified time interval after Diamox administration.

Preparing the specimen for freezing-point determination

Samples were drawn from the watch glasses, with the visual aid of a magnifying lens, into specially prepared¶ silica-quartz capillaries of 0.1 mm. I.D. The posterior chamber aqueous humor, anterior chamber aqueous humor, and plasma were each drawn into separate capillaries which in turn were inserted into oil-bearing protective tubes. Each capillary contained several samples of the same specimen, the samples being separated one from the other by a drop of oil. The protective tubes bearing the capillaries were fitted side by side into the specimen holder which held the tubes during all subsequent handling. The specimens were frozen by immersion into an alcohol-dry-ice mixture contained within a beaker or small Dewar flask outside the instrument. A quick transfer to position within the below-freezing bath was then made and microscopic observation begun.

Figure 3 shows the specimen in its initial frozen state. Gradual thawing (fig. 4) and disappearance of the frozen crystals was effected by controlled raising of the temperature until as seen in Figure 5 the last crystal approached the meniscus. The freezing-point of the given specimen is then defined as the temperature at which the last crystal disappeared behind the meniscus.

Experimental

Calibration of the instrument. To allow comparison of our results with those of other investigators it was necessary to equate

* The aqueous fluids were formerly immersed under a layer of paraffin oil to protect them from ccontact with air. It was observed, however, that if the aqueous remained in contact with the oil for as little as 10 to 15 minutes, a viscious film developed around the specimen. This formation did not occur if a specimen of NaCl solution of comparable concentration was immersed in oil or if the aqueous specimen was simply exposed to the atmosphere. With Silicone oil (Union Carbide L-45 Viscosity 100) no film developed.

† To obviate the need for heparin in the blood sample, which might affect the measured osmotic pressure, the following technique was used at first: A Luer Lock Nylon Syringe (Labtician Products Company, Hollis 23, New York) was used to withdraw arterial blood via a needle treated with "Siliclad" (a water-soluble silicone concentrate, a non-wettable material, manufactured by Clay-Adams, Inc., New York). Very frequently during centrifugation the plasma coagulated. Since the plunger in the nylon syringe was rather stiff in operation, it was assumed that some of the platelets were broken in the expulsion process from the syringe to the centrifuge tube and coagulation resulted. The nylon syringe has been replaced with a regular glass tuberculin syringe which has been Siliclad treated. In addition, all the needles, syringes, and centrifuge tubes coming in contact with the blood and plasma were Siliclad treated.

‡ To prevent hemolysis of the blood cells with a resultant red-tinged plasma color, the needle of the blood-containing syringe is placed below the oil layer in the centrifuge tube thus minimizing contact of blood with the oil.

§ Paraffin oil was used since no film formation was noted as with the aqueous humor.

¶ These capillaries were drawn to the stated diameter so that a specimen whose length equalled its diameter would have a volume of 10^{-3}mm.^{3}, specified by Ramsay as the critical maximum volume which allows for instantaneous equilibrium between bath and specimen. The capillaries were drawn from quartz tubing by James Graham, Sr., and James Graham, Jr. of the University of Pennsylvania Glassblowing Shop. A final check on capillary diameters was made using a dissecting microscope with micrometer eye piece.

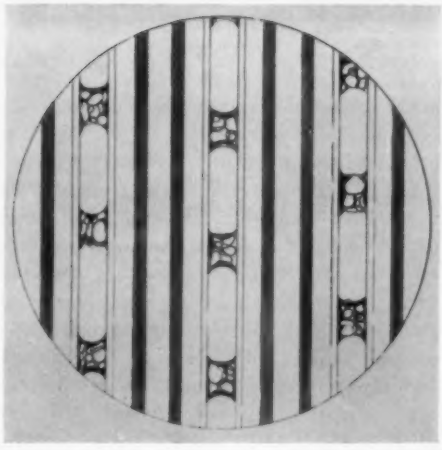

Fig. 3 (Kass and Green). Specimens as observed through microscope (X10) in their initial frozen state. (Left) Plasma. (Center) Anterior chamber aqueous (Right) Posterior chamber aqueous.

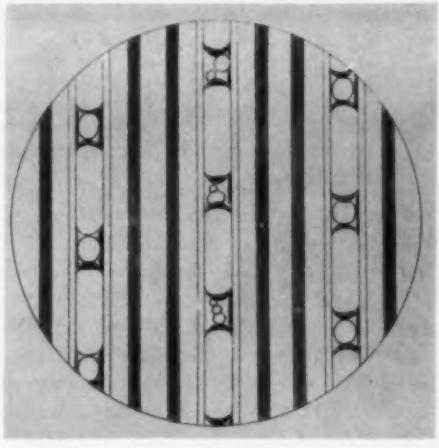

Fig. 4 (Kass and Green). Microscopic observation of specimens after partial thawing had occurred.

a given freezing-point reading with a NaCl solution of specific molality. The instrument was therefore calibrated with standard solutions of purified sodium chloride, ranging from 0.1560 to 0.1680 molality (the expected range of blood plasma and aqueous humor osmolal concentrations). Freezing-point determinations on four NaCl solutions were made numerous times, each experiment employing fresh samples from the original stock solutions.

Plasma-serum experiments. Plasma was prepared from arterial blood (as described above). An additional sample of arterial blood, withdrawn at the same time, was permitted to stand under oil for about 20 minutes to permit clotting to occur. This sample was then centrifuged and the serum withdrawn for a freezing-point determination.

Diamox experiments. As a control, the right eye of a normal untreated rabbit was tapped for posterior chamber aqueous humor and anterior chamber aqueous humor. The sodium salt of acetazolamide (30 mg./ml.) was injected at a dosage of 30 mg. per kg. of body weight into the marginal ear vein. Thirty or 60 minutes later, according to the experiment, the left eye was similarly tapped for posterior chamber and anterior chamber aqueous humor. In all cases whenever an eye was tapped a sample of blood plasma was prepared from arterial blood withdrawn immediately after paracentesis of the eye. Each animal was used only once for an experiment.

Because of the large natural variation among rabbits, each Diamox experiment had

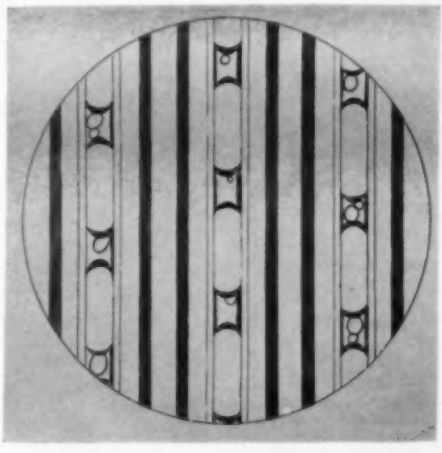

Fig. 5 (Kass and Green). Microscopic observation of anterior chamber aqueous (center specimen) approaching end-point: last crystal disappearing behind meniscus.

its own set of controls, the data of which were not used for other Diamox experiments.

Results

Instrument calibration and accuracy of measurements

From the measurements of the freezing-point depressions of standard solutions of NaCl (table 1) of concentrations from 0.1561 molal to 0.1680 molal, it can be seen that the method gave highly reproducible values for aliquots of the same stock solution with an over-all average accuracy of 0.010°C. within 95 percent confidence limits (two standard deviations). The difference of 0.010°C. was equivalent to a difference of 0.003 M NaCl/kg. H_2O in the specified concentration range. The difference between the measured values and the calculated values for the corresponding concentrations of NaCl (using constants determined by Scatchard[11]) was 0.026°C.

Aqueous humor-plasma relationships in normal rabbits (table 2).

It can be seen from the results of measurements of some 30 individual eyes that the freezing point depression of the aqueous humor of the posterior chamber (0.495°C.) was not significantly different from that of the plasma (0.490°C.). The freezing point depression of the aqueous humor of the anterior chamber (0.535°C.) was markedly greater than that of the aqueous humor of the posterior chamber as well as that of the plasma. That this difference was statistically significant was not only evident from the estimation of the student "t" test ($0.01 > P > 0.001$) but also from the fact that in all but two of the 29 comparisons the freezing-point of the aqueous humor of the anterior chamber was greater than that of the posterior chamber. These data are summarized in the form of a bar-graph (fig. 6). On the basis of concentrations the aqueous humor of the anterior chamber was

TABLE 1

FREEZING-POINTS OF STANDARD NaCl SOLUTIONS AS MEASURED ON RAMSAY-BROWN FREEZING-POINT-DEPRESSION INSTRUMENT

Experiment No.	Soln. A 0.1561 M*	B 0.1621 M	C 0.1649 M	D 0.1680 M
	(°C.)†	(°C.)	(°C.)	(°C.)
1	0.510	0.542	0.537	0.545
2	0.510	0.527	0.536	0.548
3	0.510	0.531	0.544	0.558
4	0.512	0.527	0.532	0.558
5	0.512	0.529	0.530	0.562
6	0.515	0.529	0.534	0.550
7		0.529	0.531	0.557
8			0.537	0.554
9			0.539	0.553
10			0.545	0.559
11			0.537	0 552
12			0.535	
13			0.550	
14			0.535	
Average	0.512	0.530	0.537	0.554
S.D.‡	0.0010	0.0055	0.0077	0.0045
S.E.M.‡	0.0004	0.0021	0.0021	0.0014
Calculated value§	0.536	0.557	0.567	0.577
Difference between calculated value and measured average	0.024	0.027	0.030	0.023

* Moles per 1,000 g. H_2O.
† All values of freezing-points in °C. below zero.
‡ S.D., standard deviation; S.E.M., standard error of the mean.
§ Calculated from freezing-point data of Scatchard.[11]

TABLE 2

FREEZING-POINTS OF AQUEOUS HUMORS AND BLOOD PLASMA OF NORMAL UNTREATED RABBITS

Sample	P.C.*	A.C.*	Pl.*	Sample	P.C.	A.C.	Pl.
1	0.574	0.619	0.496	16	0.485	0.493	—
2	0.545	0.630	0.472	17	—	0.496	0.485
3	0.509	0.520	0.485	18	0.465	0.512	0.484
4	0.515	0.542	0.505	19	0.466	0.615	0.478
5	0.560	0.630	0.510	20	0.550	0.535	0.490
6	0.540	0.602	0.511	21	0.464	0.500	0.478
7	0.501	0.524	—	22	0.475	0.556	0.486
8	0.477	0.510	—	23	0.455	0.497	0.488
9	0.505	0.512	0.487	24	0.513	0.615	0.497
10	0.440	0.530	0.460	25	0.450	0.495	0.465
11	0.484	0.502	0.470	26	0.500	0.525	0.488
12	0.465	0.489	—	27	0.522	0.516	0.489
13	0.465	0.495	0.495	28	0.468	0.520	0.496
14	0.493	0.517	0.498	29	0.505	0.518	0.498
15	0.478	0.493	—	30	0.477	0.530	0.528

	P.C.	*A.C.*	*Pl.*
No. of samples	29	30	25
Av. mM/1,000 g. H_2O eq. NaCl	148.5	160.5	147.0
Av. Δ (°C.)	0.495	0.535	0.490
S.D.	0.0360	0.0435	0.0141
S.E.M.	0.0066	0.0079	0.0028
"t" (A.C.-P.C.)	3.887	0.001>P	
"t" (A.C.-Pl.)	5.35	0.001>P	
"t" (P.C.-Pl.)	0.697	0.50 >P>.40	

* P.C., posterior chamber aqueous; A.C., anterior chamber aqueous; Pl., plasma.

equivalent to a 0.1605 molal solution of NaCl, while the aqueous humor of the posterior chamber and plasma were equivalent to 0.1485 molal and 0.1470 molal NaCl, respectively.

PLASMA-SERUM SAMPLES OF NORMAL RABBITS (table 3)

Before working out a suitable technique for separating plasma from whole blood without the use of heparin (spinning whole blood at the relatively low centrifuge speed of 1,000 rpm in Siliclad-treated glassware), obtaining uncoagulated plasma was a hit and miss proposition. Since serum obviated this difficulty the possibility of its use instead of plasma for the freezing-point determinations was contemplated. A comparison therefore was made of the freezing-point values of these two blood fractions.

Table 3, representing data from nine rabbits, shows that for purposes of freezing-

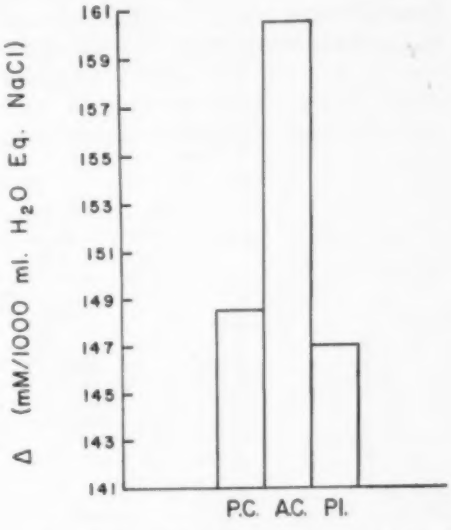

Fig. 6 (Kass and Green). Freezing-points of aqueous humors and plasma of normal untreated rabbits.

TABLE 3

COMPARISON OF FREEZING POINTS OF PLASMA AND SERUM OF NORMAL UNTREATED RABBITS

Rabbit	Plasma	Serum
1	0.495	0.488
2	0.488	0.483
3	0.492	0.492
4	0.480	0.483
5	0.465	0.465
6	0.468	0.470
7	0.490	0.490
8	0.487	0.485
9	0.498	0.496
Av. Δ (°C)	0.484	0.483
S.D.	0.031	0.031
S.E.M.	0.010	0.010
"t"	0.07	
P	P>0.90	

point studies plasma and serum may be used interchangeably.

DIAMOX-TREATED RABBITS

Comparison of aqueous humors of left and right eyes of normal rabbits (table 4). The results showed that although the freezing-point depression of aqueous humors of sister eyes of the same animal varied by as much as 0.05°C., on the average there was no significant difference between the values for the left and right eyes (0.490° and 0.490° for the aqueous humors of the posterior chambers of the left and right eyes, and 0.512° and 0.509° for the aqueous humors of the anterior chambers of the same eyes). This essential similarity between the two eyes justified the use of aqueous humors of one eye as a control for the effect of Diamox upon the aqueous humors of the contralateral eye.

Effect of Diamox upon the aqueous humors and plasma 60 minutes after injection (tables 5, 5A). It can be seen from the data in Table 5 that the freezing-point depressions of the aqueous humor of the posterior chamber and of the plasma were not significantly affected 60 minutes after the intravenous injection of Diamox. The freezing-point depression of the aqueous humor of the anterior chamber, however, was significantly less after Diamox than the control value (0.500° and 0.535° at 60 and 0 minutes, respectively). In terms of osmotic pressure, Diamox significantly decreased the osmotic concentration of the aqueous humor of the anterior chamber, but had no significant effect upon that of the posterior chamber and the plasma. These results have been summarized graphically in Figure 7.

A direct comparison of freezing-point relationships between the aqueous humors and the plasma, before and after Diamox, is given by the data in Table 5A. It is clear from the results of 10 experiments that after

TABLE 4*

COMPARISON OF AQUEOUS HUMORS OF RIGHT AND LEFT RABBIT EYES

Rabbit	P.C., Δ (°C.)			A.C., Δ (°C.)		
	O.D.	O.S.	O.S.-O.D.	O.D.	O.S.	O.S.-O.D.
1	0.512	0.480	−0.032	0.522	0.516	−0.006
2	0.507	0.487	−0.020	0.505	0.504	−0.001
3	0.500	0.495	−0.005	0.500	0.510	+0.010
4	0.465	0.490	+0.025	0.535	0.520	−0.015
5	0.510	0.500	−0.010	0.520	0.510	−0.010
6	0.492	0.497	+0.005	0.513	0.512	−0.001
7	0.443	0.498	+0.055	0.504	0.511	+0.007
8	0.491	0.470	−0.021	0.499	0.492	−0 007
Average	0.490	0.490	−0.00037	0.512	0.509	−0.0029
S.E.M.	0.003	0.003	0.0094	0.007	0.007	0.0028
Absolute difference between two eyes			0.0216			0.0071

* Right eye (O.D.) anterior chamber fluid removed after paracentesis of posterior chamber. Left eye (O.S.) then tapped in same order.

TABLE 5*

EFFECT OF DIAMOX ON FREEZING-POINTS OF AQUEOUS HUMORS AND PLASMA
(Time: 60 min. All values in terms of freezing-point depression in 0°C.)

Rabbit	Posterior Chamber			Anterior Chamber			Plasma		
	$\Delta_{0\ min.}$	$\Delta_{60\ min.}$	$\Delta_{60}-\Delta_0$	$\Delta_{0\ min.}$	$\Delta_{60\ min.}$	$\Delta_{60}-\Delta_0$	$\Delta_{0\ min.}$	$\Delta_{60\ min.}$	$\Delta_{60}-\Delta_0$
1	0.550	0.508	−0.042	0.535	0.495	−0.040	0.490	0.493	+0.003
2	—	—	—	0.556	0.505	−0.051	0.486	0.492	+0.006
3	—	—	—	0.497	0.488	−0.009	0.488	0.485	−0.003
4	0.513	0.485	−0.028	0.615	0.518	−0.097	0.497	0.495	−0.002
5	0.450	0.452	+0.002	—	—	—	0.465	0.465	0.00
6	—	—	—	0.525	0.475	−0.050	0.488	0.478	−0.010
7	0.522	0.518	−0.004	0.516	0.516	0.00	0.489	0.500	+0.011
8	0.468	0.493	+0.025	0.520	0.500	−0.020	0.496	0.488	−0.008
9	0.505	0.502	−0.003	0.518	0.508	−0.010	0.498	0.485	−0.013
10	0.477	0.496	+0.019	0.530	0.495	−0.035	0.528	0.485	−0.043
Av. mM/1,000 g. H_2O eq. NaCl	149.1	147.9	−1.2	160.5	150.0	−10.5	147.9	145.5	−2.4
Av. Δ (°C.)	0.497	0.493	−0.004	0.535	0.500	− 0.035	0.492	0.487	−0.005
S.D.	0.030	0.010		0.032	0.010		0.010	0.017	
S.E.M.	0.0096	0.0038		0.0097	0.0033		0.0030	0.0054	
"t" $_{(60-0)}$	0.388			3.431			0.806		
P	0.80>P>0.70			0.01>P>0.005			0.50>P>0.40		

* Freezing-point determinations made on aqueous humors (posterior chamber and anterior chamber) taken from right eye before Diamox administration (0 minutes) and on left eye fluids taken 60 minutes after Diamox. Control and test blood plasma (Pl.) prepared from whole blood obtained from ear arteries.

60 minutes Diamox decreased the difference in freezing-points between the aqueous humor of the anterior chamber and the plasma as well as the difference between the aqueous humors of the anterior and posterior chambers. As the data indicate, these smaller differences reflected essentially the decrease in magnitude of the freezing-point depression of the anterior chamber aqueous humor relative to those of the plasma and posterior

TABLE 5A

EFFECT OF DIAMOX ON FREEZING-POINT DIFFERENCES BETWEEN AQUEOUS HUMORS AND PLASMA
(Time: 60 min. All values in terms of freezing-point depression differences in °C.)

Rabbit	P.C.-Pl.			A.C.-Pl.			A.C.-P.C.		
	$\Delta_{0\ min.}$	$\Delta_{60\ min.}$	$\Delta_{60}-\Delta_0$	$\Delta_{0\ min.}$	$\Delta_{60\ min.}$	$\Delta_{60}-\Delta_0$	$\Delta_{0\ min.}$	$\Delta_{60\ min.}$	$\Delta_{60}-\Delta_0$
1	0.060	0.015	−0.045	0.045	0.002	−0.043	−0.015	−0.013	+0.002
2	—	—	—	0.070	0.013	−0.057	—	—	—
3	—	—	—	0.009	0.003	−0.006	—	—	—
4	0.016	−0.010	−0.026	0.118	0.023	−0.095	0.102	0.033	−0.069
5	−0.015	−0.013	+0.002	—	—	—	—	—	—
6	—	—	—	0.037	−0.003	−0.040	—	—	—
7	0.033	0.018	−0.015	0.027	0.016	−0.011	−0.006	−0.002	+0.004
8	−0.028	0.005	+0.033	0.024	0.012	−0.012	0.052	0.007	−0.045
9	0.007	0.017	+0.010	0.020	0.023	+0.003	0.013	0.006	−0.007
10	−0.051	0.011	+0.062	0.002	0.010	+0.008	0.053	−0.001	−0.054
Av. mM/1,000 g. H_2O eq. NaCl	1.8	2.4	+0.6	13.2	4.5	−8.7	11.4	2.1	−9.3
Av. Δ (°C.)	0.003	0.006	+0.003	0.039	0.011	−0.028	0.033	0.005	−0.028
S.D.	0.0349	0.0119		0.0337	0.0084		0.0403	0.014	
S.E.M.	0.0132	0.0045		0.0112	0.0028		0.0164	0.0058	
"t" $_{(60-0)}$	0.216			2.43			1.61		
P	0.90>P>0.80			0.05>P>0.025			0.20>P>0.10		

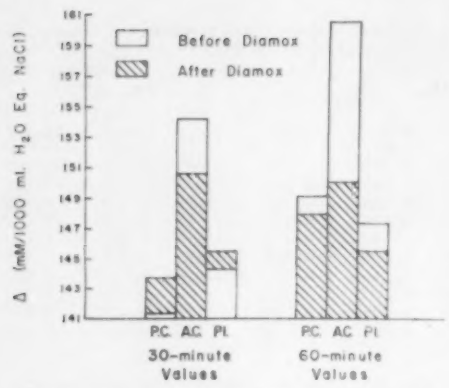

Fig. 7 (Kass and Green). Effect of Diamox on aqueous humors and plasma. Freezing-point determinations made on aqueous humors (posterior chamber and anterior chamber) taken from right eye before intravenous Diamox administration (30 mg./kg.) and on left eye fluids taken 30 or 60 minutes after Diamox. Control and test blood plasma (Pl.) prepared from whole blood obtained from ear arteries.

chamber, the latter having remained substantially constant. On the other hand Diamox had no consistent effect upon the freezing-point relationship between the aqueous humor of the posterior chamber and the plasma. In terms of osmotic pressure, the intravenous injection of Diamox significantly decreased the osmotic pressure gradient between the anterior chamber aqueous and the plasma and that between the aqueous humors of the anterior and posterior chambers but had no significant or consistent effect upon the osmotic pressure relationship between the posterior chamber aqueous and the plasma. As shown in Table 5A the decrease in the osmotic pressure gradient between the aqueous humor of the anterior chamber and the plasma 60 minutes after Diamox was equivalent to a decrease of about 8.7 mM of NaCl equivalent/Kg. H_2O.

Effect of Diamox upon the aqueous humors and plasma 30 minutes after injection (tables 6, 6A). In view of the recent observations of Green and Sawyer,[12] Gloster and Perkins,[13] and Langham[14] that Diamox elicited both physical-chemical and pharmacologic responses within the first several minutes after injection, it was deemed desirable to determine the effect of the drug upon the freezing-points at time intervals of less than 60 minutes after administration. The data of Tables 6 and 6A show clearly that Diamox had no consistent or significant effect upon the freezing-point depression of the posterior chamber aqueous humor and of the plasma, and apparently did not alter the freezing-point depression relationship between these two fluids. Regarding the aqueous humor of the anterior chamber it can be seen from the data of Table 6 that although the freezing-point depression after 30 minutes was not as large as the control value (0.502 compared with 0.514, respectively), the difference (—0.012°) was not statistically significant. Similarly while the freezing-point depression difference between the aqueous humor of the anterior chamber and the plasma was diminished by 0.0176° 30 minutes after Diamox, statistically the effect was not considered significant. These results have been summarized graphically in Figure 7.

Discussion

In the present study considerable precautions were taken in the removal of aqueous humors and arterial blood and in their handling to preserve as closely as possible the essential in vivo physiologic properties of these fluids. Furthermore, in view of the fact that the fluids to be compared were measured simultaneously under identical experimental conditions, the results of this investigation are submitted as being representative, to a significantly high degree, of the physical-chemical relationships of the aqueous humors and plasma of the normal rabbit, and of the effect of an intravenous injection of Diamox thereon.

The freezing-point depression and osmotic pressure of a solution are colligative properties, dependent *only* upon the *number* of dissolved particles, regardless of the nature of such. Accordingly, the measurements of

TABLE 6*

EFFECT OF DIAMOX ON FREEZING-POINTS OF AQUEOUS HUMORS AND PLASMA
(Time: 30 min.)

Rabbit	Posterior Chamber			Anterior Chamber			Plasma		
	$\Delta_{0\ min.}$	$\Delta_{30\ min.}$	$\Delta_{30}-\Delta_0$	$\Delta_{0\ min.}$	$\Delta_{30\ min.}$	$\Delta_{30}-\Delta_0$	$\Delta_{0\ min.}$	$\Delta_{30\ min.}$	$\Delta_{30}-\Delta_0$
1	0.440	0.487	+0.047	0.530	0.500	−0.030	0.460	0.470	+0.010
2	0.484	0.467	−0.017	0.502	0.485	−0.017	0.470	0.473	+0.003
3	0.465	0.475	+0.010	0.489	0.470	−0.019	—	—	—
4	0.465	0.498	+0.033	0.495	0.505	+0.010	0.495	0.487	−0.008
5	0.493	0.498	+0.005	0.517	0.495	−0.022	0.498	0.509	+0.011
6	0.478	0.452	−0.026	0.493	0.496	+0.003	—	0.468	—
7	0.485	0.470	−0.015	0.493	0.485	−0.008	—	0.470	—
8	—	—	—	0.496	0.533	+0.037	0.485	0.485	0.000
9	0.465	0.480	+0.015	0.512	0.507	−0.005	0.484	0.485	+0.001
10	0.466	0.490	+0.024	0.615	0.545	−0.070	0.478	0.487	+0.009
Av. mM/1,000 g. H_2O eq. NaCl	141.3	143.7	+2.4	154.2	150.6	−3.6	144.3	145.5	+1.2
Av. Δ (°C.)	0.471	0.479	+0.008	0.514	0.502	−0.012	0.481	0.485	+0.004
S.D.	0.0141	0.0141		0.0360	0.0200		0.0100	0.0141	
S.E.M.	0.005	0.004		0.011	0.006		0.004	0.005	
"t"$_{(30-0)}$	1.250			0.960			0.162		
P	0.25>P>0.20			0.40>P>0.30			0.90>P>0.80		

* Freezing-point determinations made on aqueous humors (posterior chamber and anterior chamber) taken from right eye before Diamox administration (0 minutes) and on left eye fluids taken 30 minutes after Diamox. Control and test blood plasma (Pl.) prepared from whole blood obtained from ear arteries.

the freezing-point depression of a solution may be used for the calculation of its osmotic pressure, and the relationship of the osmotic pressures of two or more fluids may be compared by the measurements of their respective freezing-point depressions.[16] By calibration of the instrument with solutions of purified NaCl over a narrow concentra-

TABLE 6A

EFFECT OF DIAMOX ON FREEZING-POINT DIFFERENCES BETWEEN AQUEOUS HUMORS AND PLASMA

(Time: 30 min. All values in terms of freezing-point depression differences, in °C.)

Rabbit	P.C.-Pl.			A.C.-Pl.			A.C.-P.C.		
	$\Delta_{0\ min.}$	$\Delta_{30\ min.}$	$\Delta_{30}-\Delta_0$	$\Delta_{0\ min}$	$\Delta_{30\ min.}$	$\Delta_{30}-\Delta_0$	$\Delta_{0\ min.}$	$\Delta_{30\ min.}$	$\Delta_{30}-\Delta_0$
1	−0.020	0.017	+0.037	0.070	0.030	−0.040	0.090	0.013	−0.077
2	0.014	−0.006	−0.020	0.032	0.012	−0.020	0.018	0.018	0.000
3	—	—	—	—	—	—	0.024	−0.005	−0.029
4	−0.030	0.011	+0.041	0.000	0.018	+0.018	0.030	0.007	−0.023
5	−0.005	−0.011	−0.006	0.019	−0.014	−0.033	0.024	−0.003	−0.027
6	—	−0.016	—	—	0.028	—	0.015	0.044	+0.029
7	—	0.000	—	—	0.015	—	0.008	0.015	+0.007
8	—	—	—	0.011	0.048	+0.037	—	—	—
9	−0.019	−0.005	+0.014	0.028	0.022	−0.006	0.047	0.027	−0.020
10	−0.012	0.003	+0.015	0.137	0.058	−0.079	0.149	0.055	−0.094
Av. mM/1,000 g. H_2O eq. NaCl	−3.0	−1.8	+1.2	9.9	5.1	−4.8	12.9	6.9	−6.0
Av. Δ (°C.)	−0.012	−0.0009	+0.0111	0.0424	0.0241	−0.0183	0.045	0.019	−0.026
S.D.	0.0113	0.0103		0.0437	0.0197		0.0434	0.019	
S.E.M.	0.0046	0.0037		0.0127	0.0066		0.0145	0.0063	
"t"$_{(30-0)}$	1.88			1.279			1.645		
P	0.10>P>0.05			0.25>P>0.20			0.20>P>0.10		

tion range (table 1) a direct comparison of the molal concentrations of the three fluids in terms of NaCl equivalents could be calculated. Such a comparison is not dependent upon any assumption concerning the nature of the osmotically active substances nor does it require any correction factor for osmotically inactive substances. Indeed, as Kinsey and Levene have shown, the osmotic pressure of a standard saline solution was not significantly affected by the presence of protein in concentrations equivalent to that present in plasma. Within the sensitivity of the present instrument (a freezing-point depression of 0.01°C. was equivalent to a difference of 0.003 moles NaCl per 1,000 gm. H_2O) the effect of the plasma protein concentration upon the freezing-point depression of the plasma could also be considered negligible.

The results of the present investigation clearly demonstrated the absence of any significant osmotic gradient between the aqueous humor of the posterior chamber and the arterial blood plasma. On the other hand, the osmotic pressure of the aqueous humor of the anterior chamber was found to be very significantly higher than that of the plasma. Furthermore, contrary to the findings of Kinsey, the results of the present investigation clearly demonstrated that the osmotic pressure of the aqueous humor of the anterior chamber was very significantly higher than that of the posterior chamber. Indeed, in view of the fact that in 27 out of 29 experiments, in which aqueous humors of both chambers of the same eye were treated under the same experimental conditions and their freezing-points determined simultaneously, the osmotic pressure of the anterior chamber fluid was significantly greater than that of the posterior chamber fluid, and, in view of the fact that the probability that this excess occurred by chance was less than one in a 1,000, the experimental findings heavily favor the likelihood that such a gradient does exist between the two chambers.

The absence of a significant osmotic pressure gradient increasing from the plasma to the aqueous humor of the posterior chamber indicates that the flow of fluid into that chamber of the rabbit eye may not be an osmotic flow. Rather it may be a direct result of a H_2O pump mechanism or associated with the active transfer of a particular ion. The recent observations of Green and Sawyer[12, 17] are in accordance with these suggestions.

The existence of a significant osmotic pressure gradient increasing from the plasma to the aqueous humor of the anterior chamber, coupled with the absence of a similar gradient between the plasma and the posterior chamber, has important implications regarding our present concepts of the dynamics of aqueous humor formation and flow. Heretofore, it has been assumed that the flow of aqueous humor out at the angle of the rabbit eye was fundamentally a reflection of the unidirectional flow of fluid from the plasma into the posterior chamber and thence through the pupillary space into the anterior chamber. The contribution of any flow directly from the plasma into the anterior chamber was not considered to be of any significance.[18, 19] The present findings, however, provide strong evidence for the existence of a significant osmotic flow directly from the plasma into the anterior chamber and suggest that the flow of aqueous humor out of the eye at the angle is composed of at least two components: (1) the undirectional flow into the anterior chamber from the posterior chamber; and (2) an osmotic flow into the anterior chamber from the plasma. This concept has provided a basis for the explanation of some of the results obtained by Green and Sawyer[12, 17] in their studies with C-14 labeled $NaHCO_3$.

It is apparent from the foregoing discussion that the hypotensive action of Diamox could not be related to the osmotic pressure relationship between the aqueous humor of the posterior chamber and the plasma, unless the drug lowered the osmotic pressure in the

posterior chamber or raised it in the plasma. On the other hand, a decrease in the excess osmotic pressure of the anterior chamber fluid relative to that of the plasma could possibly account, at least in part, for the hypotensive action of the drug. The results of this investigation on the effects of Diamox upon the osmotic pressures of the three fluids clearly showed the absence of any consistent or significant effect either upon the osmotic pressures of the plasma and aqueous humor of the posterior chamber or upon the osmotic pressure relationship between these two fluids. Consequently, it is reasonable to conclude that the hypotensive action of Diamox is not dependent upon the osmotic pressure relationship between the aqueous humor of the posterior chamber and the plasma.

The results with the aqueous humor of the anterior chamber, however, showed that the drug not only significantly decreased the osmotic pressure of this fluid in 60 minutes, but also, contrary to the findings of Levene and Tanner and Harris, significantly reduced the osmotic pressure gradient between it and the plasma. After 30 minutes of Diamox action, however, the effects were not as decisive as after 60 minutes. Thus, while Diamox in 30 minutes decreased the osmotic pressure of the anterior chamber fluid and reduced the osmotic pressure gradient relative to the plasma, because of the wide variability of the values, the degree of lowering was not considered to be statistically significant. Nevertheless, there is no doubt that the marked decrease in the osmotic pressure gradient observed after 60 minutes had been initiated at some earlier time period so that the observed decrease after 30 minutes was probably of physiologic significance. To what extent this decrease of the osmotic pressure gradient between the aqueous humor of the anterior chamber and the plasma can be correlated with the lowering of intraocular pressure and the decreased rate of flow of aqueous humor elicited by Diamox must await careful measurements of the rates of formation of aqueous humor both in the anterior chamber and posterior chamber of the rabbit eye, as discussed above.

Summary

1. The osmotic pressure relationships of the plasma and aqueous humors of the anterior and posterior chambers of the rabbit, and the effect of Diamox thereupon, were determined with the use of the micro-cryoscopic apparatus of Ramsay and Brown.

2. The ability to measure the freezing-point depressions of the aqueous humors of both chambers from the same single eye and of the plasma simultaneously under exactly the same experimental conditions, together with the high sensitivity and reproducibility of the method, are unique properties of the Ramsay-Brown apparatus.

3. The osmotic pressure of the aqueous humor of the anterior chamber in 27 out of 29 eyes was found to be markedly higher than that of the posterior chamber of the same eye, as well as that of the plasma. This excess was equivalent to 12.0 and 13.5 mM NaCl/1,000 g. H_2O, respectively.

4. There was no significant or constant difference between the osmotic pressure of the plasma and that of the aqueous humor of the posterior chamber.

5. The intravenous injection of Diamox (30 mg./kg.) had no statistically significant or constant effect upon the osmotic pressure of the plasma and the aqueous humor of the posterior chamber, or upon the osmotic pressure relationship between these two fluids in 30 and 60 minutes.

6. Sixty minutes after the injection of Diamox the osmotic pressure of the aqueous humor of the anterior chamber was decreased by an amount equal to 10.5 mM NaCl/1,000 g. H_2O. The osmotic concentration excess of the anterior chamber aqueous over that of the plasma and aqueous humor of the posterior chamber, also decreased significantly by an amount equal to 8.7 and 9.3 mM NaCl/1,000 g. H_2O, respectively. After 30 minutes of drug action,

however, although the osmotic pressure of the aqueous humor of the anterior chamber, as well as its excess over that of the plasma and aqueous humor of the posterior chamber, decreased by an amount equivalent to 4.8 and 6.0 mM NaCl/1,000 g. H_2O, respectively, the decrease was not statistically significant. Nevertheless, on the reasonable assumption that the marked decrease observed after 60 minutes was initiated earlier, the smaller decrease measured after 30 minutes was considered to be physiologically significant.

7. On the basis of the results it was suggested that the flow of aqueous humor out of the anterior chamber at the angle of the rabbit eye is composed essentially of two components: (a) the flow from the posterior chamber into the anterior chamber, and (b) a direct flow from the plasma into the anterior chamber. Furthermore, while the data do not support the concept that the flow from the plasma into the posterior chamber is an osmotic flow, the evidence strongly favors the existence of an osmotic flow from the plasma directly into the anterior chamber.

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Addendum

Similar osmotic pressure relationships were found with normal albino rabbits (14 experiments).

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AEROBIC PATHWAYS OF GLUCOSE METABOLISM IN BOVINE CORNEAL EPITHELIUM*

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INTRODUCTION

Considerable information is available concerning the glycolysis of the cornea[1,2] but not a great deal is known about the aerobic phase of glucose metabolism. Since the corneal epithelium has a significant oxygen consumption,[2] the aerobic metabolism would appear to contribute significantly to the production of biological energy. In this avascular tissue, two aerobic pathways, the tricarboxylic acid (TCA) cycle[3] and the hexose monophosphate shunt pathway,[4-6] may be functioning. In this report an attempt is made to determine how prominent a role each of these aerobic pathways plays in this tissue and how the activities of these pathways compare with those observed in other tissues.

METHODS

The epithelium was removed by scraping with a scalpel the corneas from cattle eyes. As soon as the epithelium was removed it was placed in a chilled Krebs-Ringer bicarbonate buffer aerated with 95 percent O_2 and 5.0 percent CO_2. Corneal epithelium weighing about 500 mg. was placed in a reaction vessel containing 5.0 ml. of Krebs-Ringer bicarbonate buffer and 100 μM of substrate. The incubation was run in 95 percent O_2 and 5.0 percent CO_2 for three hours at 37°C. Further experimental details were given in previous reports.[4-6]

RESULTS AND DISCUSSION

The rates of conversion of three forms of labeled pyruvate, $C^{14}H_3COCOOH$, $CH_3C^{14}OCOOH$, and $CH_3COC^{14}OOH$, to $C^{14}O_2$ were studied to obtain evidence that the tricarboxylic acid cycle (TCA) was functioning in the corneal epithelium. As might have been expected the rate at which the carboxyl ($-COOH$) carbon of pyruvate was oxidized to CO_2 was the most rapid and the methyl ($-CH_3$) carbon the least rapid. As shown in Table 1 over 12 percent of the added labeled $-COOH$ carbon of pyruvate were recovered as $C^{14}O_2$ in the course of three hours. The carbonyl ($-CO$) carbon yielded 4.54 percent as $C^{14}O_2$, while only 2.88 percent of the added $-CH_3$ carbon of pyruvate appeared as $C^{14}O_2$ in the same period. The relative rates at which the three carbon atoms of pyruvate were converted to CO_2 were expressed as ratios of $-COOH/-CH_3$ and $-CO/-CH_3$. The ratio of $-COOH/-CH_3$ from the $C^{14}O_2$ recovered was 4.36 which indicated that the rate at which the $-COOH$ carbon of pyruvate was oxidized to CO_2 was over four times as rapid as that of the $-CH_3$ carbon. The ratio of the $-CO/CH_3$ was 1.57.

It is interesting to compare the ratios observed in the corneal epithelium with those of other tissues as is shown in Table 2. Many of these ratios were determined with labeled lactate rather than with pyruvate but, since these two intermediates are metabolically interchangeable, similar results should be obtained as was the case in rat diaphragm. Since these ratios give no idea as to the activities of the TCA cycle or the aerobic process, the QO_2 is also listed and where data are available the amount of pyruvate oxidized per gram of tissue in one hour is also given. The data from $-CO$ carbon and not the $-COOH$ carbon of pyruvate was used as an index of the activity of the TCA

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TABLE 1

OXIDATION OF C^{14}-PYRUVATE TO $C^{14}O_2$ BY BOVINE CORNEAL EPITHELIUM

Experiment No.	% of Added C^{14} Recovered as CO_2 From			Ratios	
	$CH_3COC^{14}OOH$	$CH_3C^{14}OCOOH$	$C^{14}H_2COCOOH$	$\frac{-COOH}{-CH_3}$	$\frac{-CO}{-CH_3}$
1	11.68	4.32	2.82	4.15	1.53
2	14.16	4.92	3.06	4.63	1.60
3	13.10	4.76	3.12	4.22	1.52
4	12.16	4.36	3.06	3.98	1.42
5	12.00	4.58	2.68	4.49	1.71
6	12.20	4.32	2.58	4.73	1.66
Average	12.55	4.54	2.88	4.36	1.57

The results are expressed as the percentage of the added C^{14}-pyruvate recovered as CO_2 per 500 mg. of wet corneal epithelium per three hr. incubation. The specific activities used were 175 cpm/μM of $CH_3COC^{14}OOH$, 1,050 cpm/μM of $CH_3C^{14}OCOOH$ and 1,020 cpm/μM of $C^{14}H_2COCOOH$.

cycle since it is conceivable that the $-COOH$ carbon may be decarboxylated without the remaining two carbon fragments undergoing the reactions of the TCA cycle. It appears from this comparative study that regardless of the QO_2 or the activity of the citric acid cycle the $C^{14}O_2$ ratios of $-COOH/-CH_3$ and $-CO/-CH_3$ are remarkably similar with the exception of the lens. The similarity in the ratios of the corneal epithelium and the liver is particularly striking. Although the liver has a much higher TCA activity than the corneal epithelium as evidenced by the oxidation of 28 μM/gm./hr. of pyruvate as compared to 3.0, the ratios are almost identical. The results with variously labeled pyruvate suggest strongly that the TCA cycle is functioning in the corneal epithelium. The slightly higher $-COOH/-CH_3$ ratios observed in liver and corneal epithelium than those of other tissues may be due to some of the acetyl Co A molecules formed after decarboxylation of pyruvate undergoing reactions (for example, fatty acid synthesis) other than those of the TCA cycle. There is an

TABLE 2

$C^{14}O_2$ RATIOS OF THE CARBON ATOMS OF PYRUVATE OR LACTATE

Tissues	Type of C^{14} Substrate	Reference	$\frac{-COOH}{-CH_3}$	$\frac{-CO}{-CH_3}$ or $\frac{-CHOH}{-CH_3}$	Q_{O_2}	Pyruvate Oxidized μM/gm./hr.
Diaphragm (rat)	pyruvate	7	1.68	1.38	6	8
	lactate	8	1.74–2.1	1.21–1.48		
Kidney (rat)	lactate	8	2.19–2.53	1.38–1.53	21	
Liver (rat)	lactate	8	3.54–4.36	1.54–1.67		
	lactate	9	4.57	1.65	12–17	
	pyruvate	10				28
Mammary gland (rat)	lactate	11	3.4	1.68		
Corneal epithelium (cattle)	pyruvate		4.36	1.57	4	3
Corneal epithelium (rabbit)		2			6.3	
Lens (cattle)	pyruvate	12	22	3.0	0.1	0.07

The Q_{O_2} data were obtained from White, et al.,[12] with the exception of the ocular tissues.

indication that in most tissues the relative rates of oxidation of the carbon atoms CH_3:CO:COOH of pyruvate are approximated by the values of 1:1.5:2-4. It appears that, regardless of the activity of the aerobic process, the characteristics of the TCA cycle and the associated electron transmitting system are such that the rates of oxidation of the three carbons of pyruvate seem to fall into a definite pattern. The exception, thus far, seems to be the lens where decarboxylation of −COOH carbon occurs to some extent but the other two carbon atoms of pyruvate are not oxidized at a rate corresponding to that observed in other tissues. Whether this is what occurs in tissues with an extremely sluggish TCA cycle or whether the decarboxylation process is coupled to a set of reactions other than the TCA cycle are problems currently being investigated.

The other aerobic pathway which has been demonstrated to occur in the corneal epithelium is the hexose monophosphate shunt mechanism or the pentose phosphate cycle. The corneal epithelium has the enzymes needed to carry out the selective oxidation of the carbon-1 (C-1) atom of glucose and the recycling of the pentose phosphate formed to synthesize a new hexose phosphate molecule.[4] Experiments with glucose labeled in the C-1 and C-6 position provide evidence suggesting that the corneal epithelium has one of the most active shunt mechanisms as is apparent when the data from this tissue are compared with others.

In Table 3 is a summary of a comparative study of 1-C^{14} and 6-C^{14} glucose metabolism in various tissues. The results are expressed as the ratio of C-1/C-6 incorporation into lactate, fatty acids, and CO_2. The C-1/C-6 ratio of incorporation into lactate or fatty acids gives the fraction of glucose metabolized via the glycolytic pathway of Embden-Meyerhof and the difference from 1.0 gives the fraction of glucose metabolized by the shunt mechanism. Thus, in liver 86 percent of glucose is metabolized by the Embden-Meyerhof pathway and 14 percent by the shunt mechanism. The corneal epithelium has one of the highest shunt activities with 35 percent of the glucose metabolism being carried out by this mechanism. Only in the lactating mammary gland of the rat has a higher shunt activity been

TABLE 3

GLUCOSE 1-C^{14} AND 6-C^{14} METABOLISM IN VARIOUS TISSUES*

Tissues	Ratio of C_1/C_6 in		Reference
	Lactate or Fatty Acids	CO_2	
Diaphragm (rat)		1.0	Bloom and Stetten[14]
Kidney (rat)		1.0	Bloom and Stetten[14]
		1.2	Wenner and Weinhouse[15]
Retina (cattle)	0.90	1.2	Futterman and Kinoshita[16]
Heart (rat)		1.2	Wenner and Weinhouse[15]
Liver (rat)		2.8	Bloom and Stetten[14]
	0.86	2.5	Katz, et al.[17]
	0.86	1.4	Ashmore, et al.[18]
Liver (mouse)		1.5	Agranoff, et al.[19]
Hepatoma (mouse)		2.4	Agranoff, et al.[19]
Hepatoma (mouse)	1.00	3.1	Wenner and Weinhouse[15]
Hepatoma (rat)	0.82	3.0	Wenner and Weinhouse[15]
Spleen (rat)		2.4	Kit[20]
Thymus (rat)		1.6	Kit[20]
Adenocarcinoma (mouse)	0.79	3.1	Wenner and Weinhouse[15]
Corneal Epithelium (cattle)	0.65	6.7	Kinoshita, et al.[5]
Cornea (cattle)		10.0	Kinoshita[12]
Lactating Mammary Gland (rat)	0.42	18.0	Abraham[11]
Lens (rabbit)	0.86	44.0	Kinoshita and Wachtl[21]

* Most of the data presented here were obtained from the article by Ashmore, Karnovsky, and Hastings.[22]

observed. Table 3 includes tissues which rely primarily on the TCA cycle for the production of CO_2 from glucose like the diaphragm, kidney, heart, and retina. In these tissues the ratio of C-1/C-6 in CO_2 is near unity. In contrast to these tissues, the lens appears to occupy an unusual position with an extremely high ratio of 44. The high ratio in CO_2 is apparently a result of the sluggishness of the TCA cycle since the lens does not exhibit an unusually high shunt activity as indicated by the ratio of C-1/C-6 in lactate. The lactating mammary gland and the corneal epithelium both show a high selective oxidation of C-1 of glucose with a relatively high activity of the shunt mechanism. It may be concluded that the outstanding characteristic pattern of metabolism in the corneal epithelium is that a relatively large fraction of glucose is metabolized by the shunt mechanism.

In regard to the pentose phosphate cycle, we explored the possibility of other carbon atoms than C-1 of glucose being oxidized by this mechanism in the corneal epithelium. To approach this problem the rates at which 2-C^{14} and 6-C^{14} glucose are oxidized to $C^{14}O_2$ were determined. The rationale behind this approach is that if the catabolism of glucose proceeds via the TCA cycle then the $C^{14}O_2$ ratio of the C-2/C-6 of glucose should be identical with the $C^{14}O_2$ ratio of $-CO/-CH_3$ of pyruvate. This appears reasonable since in glycolysis the C-2 of glucose becomes the carbonyl carbon of pyruvate and C-6 of glucose the methyl carbon. A higher ratio in the $C^{14}O_2$ derived from the 2-C^{14} and 6-C^{14} glucose than that obtained for pyruvate would suggest that the C-2 of glucose is oxidized by a mechanism other than the TCA cycle.

The results which are summarized in Table 4 indicated that the rate of oxidation of C-2 of glucose was much more rapid than could be accounted for by the TCA cycle. The $C^{14}O_2$ ratio of C-2/C-6 of glucose gave a value of 4.28 which was much higher than the $-CO/-CH_3$ ratio of pyruvate of 1.57 (table 1). In the corneal epithelium it would appear that the pentose phosphate cycle is sufficiently active to permit the oxidation of the C-2 of glucose via the alternative pathway. For this to occur, there is first the preferential oxidation of C-1 of glucose to yield CO_2 and pentose phosphate. Then two molecules of pentose phosphate interact to undergo a series of reactions leading to the synthesis of a new hexose phosphate molecule. The C-2 of the original glucose moiety becomes the C-1 of the newly formed hexose phosphate molecule. The dehydrogenation reactions of the shunt mechanism which follow then convert this carbon to CO_2. It is possible to approximate how much of the C-2 of glucose is oxidized via the two possible pathways. The amount of C-2 of glucose oxidized via the citric acid cycle can be estimated by taking the amount of C-6 oxidized

TABLE 4

OXIDATION OF C^{14}-GLUCOSE TO $C^{14}O_2$ BY CORNEAL EPITHELIUM

Experiment No.	% of Added C^{14} Recovered as CO_2 From		Ratio C_2/C_6
	Glucose 2-C^{14}	Glucose 6-C^{14}	
1	2.49	0.59	4.22
2	2.66	0.68	3.92
3	2.23	0.50	4.46
4	2.25	0.57	3.96
5	2.41	0.52	4.64
6	2.40	0.54	4.45
Average	2.41	0.57	4.28

The results are expressed as the percentage of added C^{14} glucose recovered as CO_2 per 500 mg. of wet corneal epithelium per three hr. of incubation. The specific activities used were 835 cpm/μM of glucose 2-C^{14} and 855 cpm/μM of glucose 6-C^{14}.

and multiplying by 1.57, the ratio established by the pyruvate experiments. The estimation reveals that 0.9 percent of the 2.41 percent of the C-2 oxidized is via the TCA cycle and the remainder of $C^{14}O_2$ produced is presumably derived from the shunt mechanism. This suggests that 1.51 percent of 2.41 percent or 63 percent of the C-2 oxidized occurs via the shunt mechanism. It is assumed that all of the C-6 of glucose is oxidized by the TCA cycle. The approximation also depends on the assumption that the specific activities of the lactate or pyruvate formed from the C-2 and C-6 labeled glucose are equal and little randominzation of carbon atoms occurs. From the two experiments in which lactate was isolated and the C^{14} incorporated studied it appears that the specific activity of lactate from C-6 labeled glucose was slightly higher than that from the C-2 labeled glucose. The carboxyl groups of lactate from C-2 labeled glucose did not yield significant quantities of C^{14}. These findings would not appreciably alter the approximation.

These studies reveal that the corneal epithelium possesses a rather unique pattern of glucose metabolism in that it seems to depend more heavily on the shunt mechanism than the other tissues. The TCA cycle undoubtedly is functioning but appears to play a limited role as evidenced by the large accumulation of lactate in vivo and by the relatively small amount of pyruvate oxidized by this tissue. If the shunt plays such a prominent role in the glucose metabolism one wonders if any biological energy is derived in this process. Evidence from studies in other tissues seems to suggest that the TPNH formed in the shunt process is not involved in energy production but rather as the electron donor for synthetic reactions. It would seem that in the corneal epithelium, where a relatively large fraction of glucose is metabolized via the shunt mechanism, such a scheme would be disadvantageous if it were not geared to energy producing mechanisms. Perhaps alternate mechanisms like the coupling of the shunt dehydrogenases with lactic acid dehydrogenase[6] may serve to derive biological energy for this tissue.

Summary

A study of the aerobic phase of glucose metabolism in bovine corneal epithelium was undertaken. From the relative rates of oxidation of three carbon atoms of pyruvate it would appear that the tricarboxylic acid cycle is functioning in this ocular tissue. The hexose monophosphate shunt pathway appears to play a more prominent role in this tissue than in others. The results seem to suggest that the majority of the carbon-2 atoms of glucose are oxidized via the shunt pathway.

243 Charles Street (14).

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LENS PROTEINS*

II. The effect of pH on alpha crystallin

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In the past few years the lens proteins have been subjected to analysis by physical chemical means.[1-3] Of these studies, only two[1,2] have furnished detailed information about the size and shape of alpha crystallin. Its molecular weight is 840,000 according to the studies of Orekhovich, et al.,[1] and 930,000 according to Resnik.[2,4] These values were calculated from the sedimentation and diffusion coefficients. Both laboratories found that alpha crystallin does not display any appreciable concentration dependence in its rate of sedimentation. In an earlier report[2] it was thought that this behavior appeared to be anomalous. Recent studies of alpha crystallin, some of which will be presented at this time, have revealed that its sedimentation behavior is not contrary to theories of the relationship between sedimentation and concentration. In addition, the hydrodynamic properties of alpha crystallin have been noted to be appreciably affected by pH and ionic strength. The size and shape of alpha crystallin is discussed in the light of this information.

Methods and Material

Whole calf lenses were homogenized and then fractionated by the procedure previously described.[2] The ammonium sulfate fractionation method of Orekhovich[1] gave similar results and was used only in the early parts of this study. It should be noted that alpha crystallin isolated by both methods may contain an admixture of beta crystallin.[1,4]

Protein solutions were prepared at various

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pH values either by adding 0.1 M HCl or 0.1 M NaOH, or by using appropriate buffers. The protein concentrations of these solutions were measured either by drying them to constant weight at 105° to 110°C. or by their optical density at 280 mμ.

The viscosity of aqueous solutions of alpha crystallin was measured at 30 ± 0.01°C., using Ostwald-Fenske viscometers with flow times of about 240 seconds. Values of the reduced specific viscosity were extrapolated to infinite dilution to obtain the intrinsic viscosity at each pH.

Ultracentrifuge experiments were performed at room temperature in a Spinco Model E ultracentrifuge at a speed of 59,780 rpm. Sedimentation coefficients were corrected to 20°C. Electrophoresis experiments were carried out using a Spinco Model H electrophoresis apparatus at a bath temperature of 0.9° ± 0.01°C. A Beckman Model G pH meter was used to measure the pH of solutions. The apparent specific volume was determnied pyconometrically at 20°C.

Results

Viscosimetry

Figure 1 illustrates the pH dependence of the intrinsic viscosity of alpha crystallin. Its hydrodynamic properties are altered below pH 4.5. At an ionic strength ≪0.01 the

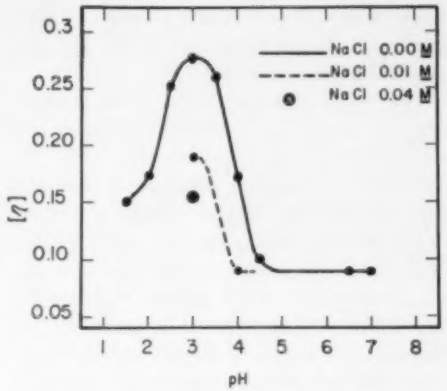

Fig. 1 (Resnik and Kenton). The effect of pH and salts on the intrinsic viscosity of alpha crystallin.

intrinsic viscosity reaches a maximum value between pH 3.0 and 3.5 while below pH 3.0 it decreases but does not return to a value as low as at pH 4.5. An increase in viscosity at low ionic strength (fig. 1) might be due to a swelling of the molecule;[5] however, in this instance, the slopes of the plots of reduced specific viscosity (fig. 2) are either zero or positive. Therefore, it seems unlikely that an electrostatically induced swelling could account for the increase in viscosity. No change was detected in the apparent specific volume. A value of 0.73

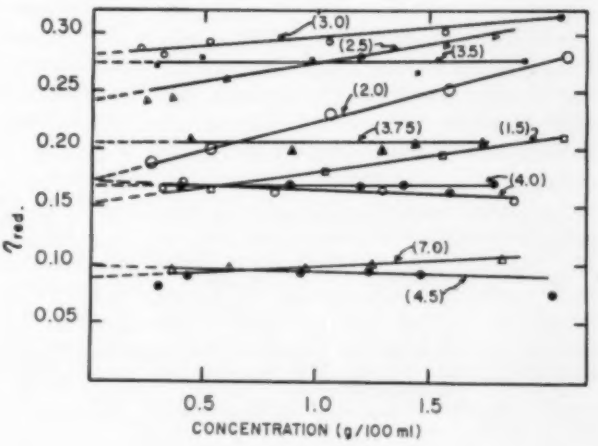

Fig. 2 (Resnik and Kenton). Plots of the reduced specific viscosity, at various pH values (in parentheses), extrapolated to infinite dilution.

ml./g. was obtained in the absence of salts at pH 3.0, in 0.1 M glycine at pH 3.0, and in 0.1 M phosphate at pH 7.0.

The positive slopes at low ionic strength (fig. 2) are probably manifestations of the electroviscous effect, which should be reduced or eliminated at higher ionic strength. The intrinsic viscosity decreased at pH 3.0 in the presence of higher salt concentrations (fig. 1). It was not possible to obtain any information in 0.1 M sodium chloride because the viscosity of these solutions increased with time at 20°C. and at 30°C. This time dependent increase in viscosity in 0.1 M salts appears to be a complex reaction and will be studied separately.

Sedimentation

In addition to the increase in viscosity at acid pH and low ionic strength, the sedimentation coefficient decreases below pH 4.5 (table 1). The molecule splits into two components as evidenced by the appearance of two boundaries in 0.1 M glycine at pH 3.0 or 3.4 (fig. 3). In this same pH range, in either 0.1 M sodium chloride or 0.5 M glycine, only one boundary is seen. No cleavage is observed at pH 4.3 to 4.4 as the concentration is lowered. To obviate the possibility that an admixture of beta crystallin might influence the sedimentation pattern, the following experiment was conducted. A homogenate of whole lenses was subjected to electrophoresis at pH 8.3, in veronal buffer, until the alpha crystallin component was completely resolved. A sample of electrophoretically pure alpha crystallin was removed from the ascending boundary and dialyzed versus pH 3.0 in 0.1 M glycine buffer for 15 hours. The ultracentrifuge pattern of this solution also revealed two sedimenting boundaries.

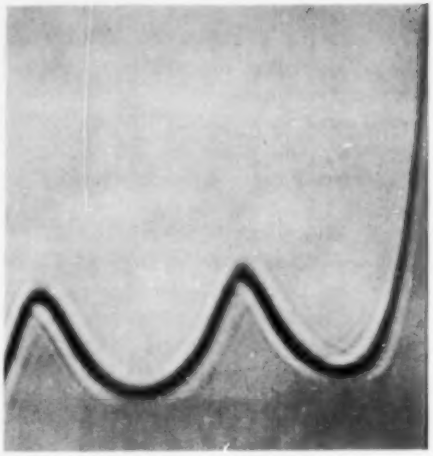

Fig. 3 (Resnik and Kenton). Sedimentation pattern of one-percent alpha crystallin in 0.1 M glycine, pH 3.1, after 99 minutes at 59,780 rpm.

To rule out the possibility that the pH 5.0 and 12-percent alcohol fractions might account for these results, the pH 5.0 and 12-percent alcohol insoluble material were separated from each other. Solutions of these fractions in 0.1 M glycine at pH 3.0 gave identical results. From these experiments it appears that the splitting observed is due to electrostatic factors which prevail when the ionic strength is not high enough.

Sedimentation data of alpha crystallin in 0.2 M, pH 7.0, phosphate buffer are listed in Table 2. By extrapolating these results to infinite dilution a value of 18×10^{-13} is ob-

TABLE 1

The effect of pH and solvent on the rate of sedimentation of alpha crystallin

pH	Solvent	$S_{20} \times 10^{13}$
7.0	0.1 *M* NaCl	17–18
6.0	0.2 *M* Ac^-	21
4.4	0.16 *M* Ac^-	21.5
3.4	0.1 *M* gly.	14.6
3.1	0.1 *M* NaCl	16.5

TABLE 2

Sedimentation data of alpha crystallin in 0.2 M, pH 7.0, phosphate buffer

Concentration (%)	$S_{20} \times 10^{13}$
1.53	16.4
1.22	17.3
1.01	17.5
0.79	17.5
0.59	17.7
0.39	17.8

TABLE 3

THE SIZE AND SHAPE OF ALPHA CRYSTALLIN

Hydration	f/f_0[1]	[2]	Prolate		Oblate	
			2a (Å)	2b (Å)	2a (Å)	2b (Å)
	1.78		778	52	17	346
20%		8.2	466	67	26	264
30%		7.4	422	70	29	266

[1] Calculated from the diffusion coefficient of 1.78×10^{-7} (2).
[2] An intrinsic viscosity of 0.076 obtained in 0.1 M phosphate, pH 7, was used.

tained for the sedimentation coefficient. According to sedimentation theory[6] k_s in the equation $S_{corr} = S_{obs} \quad [1 + (1 + k_s) \quad \phi]$, where ϕ is the volume fraction of sedimenting particles, k_s should be about 4.0 for sedimenting spheres. From the plot of 1/S versus concentration using the data in Table 2, k_s is 3.0. Since asymmetric molecules would not be expected to have the same value for k_s as spherically shaped ones, this figure is in general agreement with theory.

ELECTROPHORESIS

Between pH 4.0 and 8.3, solutions of 0.8-percent alpha crystallin migrate as a single boundary. At pH 3.0 in 0.09 M glycine buffer, the patterns are not enantiomorphic. The ascending boundary is hypersharp compared to the very broad descending boundary. Such anomalies are not uncommon in moving boundary electrophoresis when the protein concentration is too high or the ionic strength is too low.[7] Furthermore, electrophoretic studies of proteins indicate that some buffers, particularly those containing amino acids, can produce artefacts at low pH.[8] When the ionic strength of solutions of alpha crystallin in 0.1 M glycine at pH 3.0 is increased to 0.15, by the addition of sodium chloride, the boundaries are more enantiomorphic. There is no evidence of more than one component. These results are consistent with those obtained in the ultracentrifuge in that no splitting is observed at pH 3.0 if the ionic strength is sufficient to overcome the effects of electrostatic factors.

SIZE AND SHAPE

The axial ratios and molecular dimensions of alpha crystallin calculated from the viscosity data and the diffusion coefficient are summarized in Table 3. These data illustrate the effect of hydration on the asymmetry of alpha crystallin. Information obtained from the diffusion data does not include the influence of hydration. Since proteins exist in the solvated state, a more realistic interpretation is one in which this factor is considered. From the intrinsic viscosity assuming varying degrees of hydration, it is apparent that solvation alone may account for a large part of the asymmetry indicated by the diffusion data.

DISCUSSION

Investigations of the effect of environment on proteins have revealed that under the proper conditions at acid pH changes will occur in one or more of the physical properties. Recent studies of ovalbumin and serum albumin, for example, demonstrate the different effects of salts and pH which may be observed on viscosity,[5] volume,[9] and optical rotation[5, 9] of these proteins in solution. Below the isoelectric point volume changes have been observed which may or may not be accompanied by changes in other physical properties. The ramifications of this with regard to protein structure are discussed by Kauzmann.[9] The total volume change is influenced by reduction of electrostriction, due to neutralization of the carboxylate ion,[10, 11] and by the compactness of the molecule. In the absence of complicating

factors, neutralization of the carboxylate should result in a net volume increase. In this work no increase was detected at pH 3.0, the region where the viscosity change was maximum. Using a more refined technique it should be possible to determine the pH at which such volume changes as would be expected might occur.

The observed salt and pH effects on viscosity may involve, or reflect, the degree of aggregation of alpha crystallin. This is revealed in the sedimentation studies. Further information which may be used to substantiate this interpretation comes from preliminary electron microscope observations of the alpha crystallin. There are marked differences in the appearance of samples of this protein obtained by preparative electrophoresis or by chemical means. Elongated and smaller spheroid structures have been observed in these preparations, respectively. The possibility that separate units may combine to a filamentous element is suggested. These observations are of further significance for the following reason. It is not possible to decide whether a molecule is best represented by an equivalent prolate or oblate ellipsoid of revolution from viscosity data alone.[12] The electron microscope data suggest that alpha crystallin may be represented as a prolate ellipsoid of revolution.

Summary

In this investigation it has been shown that alpha crystallin may undergo various changes under certain conditions of pH and ionic strength. The molecule dissociates into two units at pH 3.0 at low ionic strength. Solutions of alpha crystallin exhibit an increase in viscosity below pH 4.5. The sedimentation behavior of alpha crystallin at pH 7.0 is in general accord with some of the concepts of the relationship between concentration and the rate of sedimentation.

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RANDOM OCCURRENCE OF HERPETIC DISCIFORM KERATITIS IN RABBITS*

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INTRODUCTION

Disciform keratitis is a term introduced by Fuchs,[1] in 1901, to describe a central opacification of the cornea that was most often seen in late complications of herpes simplex keratitis. The condition in human subjects is one that is characterized by a central opacity that extends deeply into the corneal parenchyma along with an associated thickening of the cornea. The peripheral tissue usually remains clear and undisturbed. Externally the opacity assumes a discoid appearance, hence the name disciform keratitis. Often there is a concentric structure to the discoid opacity. In the human the inflammation is nonsuppurative and runs through a benign chronic course. In more recent years, however, a more severe form has become evident in which stromal necrosis, scar formation, and uveal inflammation are factors which may further complicate the classical lesion. More detailed descriptions of the disease may be found in textbooks by Duke-Elder,[2] and Thomas,[3] and in recent publications.[4-17]

The purpose of this paper is to describe the type of lesion that occurs in the rabbit eye and which seems to be the experimental counterpart of the human disease. Opacification of the corneal stroma in the rabbit apparently is an infrequent sequela of herpetic ulcers of the epithelium. Out of 290 rabbit eyes infected with herpes simplex virus a total of 24 opacities simulating disciform keratitis were seen. These included an irregularly shaped lesion as well as the discoid type and all were not centrally located. Nevertheless, they all had features in common which suggested that a single disease entity was being observed. Thus the term "disciform" was retained in this study even though strict observance of its descriptive meaning was not maintained. The work also includes a number of other opaque eyes that suffered from secondary bacterial infections during the course of herpetic keratitis. These, along with the abovementioned, were tested for living virus and the data obtained corroborates with similar poor isolation results from human corneas. Quantitative data on the infectious dose of virus in rabbits suggested that this low incidence of isolation was due in part to a low level of virus in the inoculum prepared from disciform corneas.

A variety of etiologic agents are known to cause disciform keratitis in humans. These include viruses, bacteria, and noninfectious insults, examples of which are: herpes simplex, vaccinia, herpes zoster, varicella, mumps, syphilis, and hydrogen gas.[2,3] Trauma is a factor that has also been reported but more likely it merely offers a portal of entry for an infectious agent, which has, in many cases, remained undetected.

The random occurrence of disciform keratitis in both humans and animals suggests that in those instances, a secondary influence(s) comes into prominence at some stage of the herpetic infection and thereby stimulates an alteration in the normal pathologic pattern. The mechanism by which this alteration comes about is little understood at present and is considered in some detail in the comment section of this paper. One suggestion proposed by Braley[4] is that a hyper-

* From the Research Department of the Wills Eye Hospital. This investigation was supported in part by Research Grants 2B-5076(C) and B-1516 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, Bethesda, Maryland. Presented before the Eastern Section, New York, November, 1958.

sensitivity of the cornea to herpes virus develops in the susceptible individual and that the virus combines with local and circulating antibodies to produce a hypersensitive reaction. If such is the case then it is to be supposed that the virus or a viral antigen must, at some stage of the development of disciform keratitis, penetrate into the stromal tissues and there participate in the hypersensitive reaction. The only evidence offered is the seeming efficacy of steroid treatment, in some cases, if administered at a critical moment of the onset of the corneal opacity. Substantial evidence for this hypothesis is still lacking.

Different workers have made many attempts to demonstrate the presence of virus in the stromal tissue of human cases of disciform keratitis. Tissue obtained for this work has included corneal scrapings and sections from keratoplasty operations. Hanna, et al.,[5] recovered herpes simplex virus from two out of 32 cases of human disciform keratitis using the chorioallantoic membrane and Hela cell culture methods of isolation. Braley[4] isolated the virus on three occasions from keratitis metaherpetica, a chronic herpes infection, using mouse brain passage followed by tissue culture inoculation. Braley believes that sufficient local antibody is obtained with the corneal scrapings which neutralizes free virus that might be present and that successive passages are necessary to increase the virus concentration. Hogan[6] reported two unsuccessful attempts to demonstrate virus in corneas obtained during keratoplasty by cultivation on Hela cells and chorioallantoic membranes of chick embryos. Other isolations have been reported by Bencini[7] and Gundersen.[8]

The demonstration of disciform keratitis in laboratory animals has not been much reported in the literature in the past. Gruter[9] was, perhaps, the first one to discuss the condition in rabbits and reported a total of six such lesions which he noted over a period of 16 years. Five of the disciform keratitis lesions resulted from a total of 15 herpetic dendritic ulcers while a single inoculation of a rabbit cornea with vaccinia virus also resulted in a disciform-type lesion. The pathology of this series was not described in detail. Schirmer[10] also makes mention of a stromal involvement in the guinea pig cornea after inoculating the surface with vaccinia virus.

The study of herpes simplex virus and especially its effect on ocular tissues has attracted increasing attention from many workers since the end of World War II. Much of this work resulted from the increasing severity of the disease during this period; complications developed that often produced stromal necrosis and more severe involvement in iridocyclitis and secondary glaucoma. Experimental study in 1951 by Leopold, et al.,[11] Hallett, et al.,[12] and Ormsby, et al.,[13] and later by Thygeson[14] and Kimura[15] and associates, demonstrated that the therapeutic application of corticosteroids had a deleterious effect on the course of herpes simplex keratitis; this evidence plus accumulating clinical experience led eventually to the impression that the altered pattern from the earlier benign form of the disease to the more severe type was associated with the availability and widespread use of corticosteroids in the postwar period.[16, 17]

More basic knowledge concerning the mechanism of formation of disciform keratitis will make possible more effective and rational approaches to the therapy of this disease. It is to be hoped that means will eventually be found to produce a herpetic disciform keratitis at will in some suitable animal.

Materials and Methods

Young albino and pigmented rabbits weighing approximately 1.5 to 2.5 kg. were used. Their eyes were anesthetized by topical application of tetracaine (pontocaine, one percent) and the corneal epithelium was then mechanically abraded over a central area that was approximately 5.0 to 6.0 mm. in diameter. Care was taken not to damage the underlying stromal tissue during this

procedure. Inoculation with active herpes simplex virus was carried out by either of two procedures depending upon which of several strains of virus was used. One strain of virus, the HF strain,* was maintained by passage on the chorioallantoic membranes of embryonated hens' eggs. These membranes were stored at −20°C. in 50-percent glycerine and used as needed. Only those membranes which had been inoculated with a standard, "undiluted" or "10^{-1}" virus suspension, and had developed confluent pock lesions, were utilized for stock passage or corneal inoculation. Such membranes were ground in a mortar with an alundum abrasive, resuspended in buffer-gelatin solution† (1.0 ml. per chorioallantoic membrane), centrifuged at 1,500 rpm to sediment abrasive and cellular debris, and the virus laden supernatant fluid used as "undiluted" inoculum. For rabbit passage 0.01 ml. of this fluid was placed on the abraded cornea and contained approximately 10^6 to 5×10^6 pock-forming units when titrated out on eggs. Inoculation of eggs was carried out by removing the shell over the air sac of 13-day-old eggs, peeling off the shell membrane‡ with an iris forceps thereby exposing the chorioallantoic membranes which could then be treated with 0.1 ml. of virus containing fluid. Harvesting of the chorioallantoic membrane took place after two days' incubation at 35° to 36°C. at which time the visible portion of the chorioallantoic membrane was snipped out and either used immediately or stored as has already been described.

A second virus (the Petito strain‖) was isolated in this laboratory from a patient with a dendritic ulcer by passage of corneal epithelium scrapings directly to the abraded corneas of a rabbit. Scrapings were placed in 0.5 ml. of chilled brain heart infusion broth and corneal inoculation was made within 90 minutes. Stock passage of this strain was from rabbit to rabbit simply by transfer of epithelial scrapings to the abraded corneas of acceptor animals. Scrapings were best taken from the second to sixth day after inoculation; material taken later than this usually contained insufficient amounts of active virus. Stock material could be preserved by storage of whole corneas in 50-percent glycerine at −20°C. and virus maintained in this condition for as long as 140 days was found to be viable for the rabbit eye.

Two other virus strains were isolated from humans; one with a dendritic keratitis (Bulkley strain) and one with herpes labialis (Notaro strain). The former was passed in the same manner as the Petito strain but could only be maintained after a few rabbit passages. The Notaro strain of virus was isolated from vesicle fluid and first grown on eggs before rabbit transfer was started.

Results

As mentioned earlier, the disciform keratitis lesion produced in rabbits in this work was a random occurrence, that is, all animals were inoculated with topical application of virus to the scarified corneal epithelium and the ensuing infection allowed to run its course without any experimental interference. The average infection varied in intensity from a mild branching dendritic ulcer to one that caused large de-epithelized patches with conjunctivitis and sometimes pannus formation. The majority of these were self limited infections and healed with-

* The HF strain is an egg-adapted virus that has been through more than 380 serial transfers. It was kindly supplied to us by Dr. T. F. McNair Scott of Children's Hospital, Philadelphia.

† The buffer-gelatin solution prepared according to the method of McNair Scott[28] was supplemened with 0.06 M lysine, an inhibitor of alkaline phosphatase which inactivates herpes simplex virus (Amos[29]).

‡ The removal of the shell membrane can be facilitated by placing on it a drop of 50 percent glycerine with a sterile cotton applicator just prior to its removal. Treatment of three to four eggs at a time in this manner facilitates the process by permitting a clean removal of the membrane while it is yet loose and wet.

‖ Material for this strain was kindly supplied by Dr. Richard Lubowitz of Graduate Hospital, Philadelphia.

TABLE 1

OCCURRENCE OF DISCIFORMLIKE KERATITIS IN RABBITS RECOVERING FROM PRIMARY DENDRITIC ULCERS

Rabbit Eye	Virus Strain	Characteristics of "Disciform" Corneas				Time† of Occurrence		
		Stromal Opacity	Thickening	Anesthesia	Vascularization	Of Vascularization	Of Opacity	Of Exudate
						(days)	(days)	(days)
862 O.S.	HF	+	+	−	+	10	12	N
894 O.S.	HF	+	0	+	+	6–12	14	N
971 O.D.	HF	+	+	+	+	6	7	N
971 O.S.	HF	+	+	+	+	6	7	N
988 O.S.	HF	+	+	+	+	8	8	N
309 O.S.	HF	+	+	+	+	< 7	>10	N
900 O.D.*	Petito	+	+	+	+	9–11	9	3–8
901 O.D.*	Petito	+	+	−	+	9–11	9	3–8
922 O.D.	Petito	+	+	+	+	10	10	2–7
939 O.S.	Petito	+	+	+	+	13	13	2–12
942 O.S.	Petito	+	+	+	+	11	7–11	N
948 O.S.	Petito	+	0	+	+	<10	<10	3–5
957 O.S.	Petito	+	+	0	+	<10	<10	2
982 O.D.	Petito	+	+	−	+	< 9	10	6–9
982 O.S.	Petito	+	+	−	+	10	10	6–9
999 O.D.	Petito	+	+	+	+	7	8–10	N
475 O.D.	Petito	+	+	+	+	4	10	N
475 O.S.	Petito	+	+	+	+	4	10	N
1964 O.D.*	Bulkley	+	+	+	0	—	12–14	3–8
1964 O.S.*	Bulkley	+	+	+	0	—	12–14	4–8
210 O.D.*	Notaro	+	+	+	+	<12	13–31	N
210 O.S.*	Notaro	+	+	+	+	<31	13–31	N
217 O.D.	Notaro	+	+	+	+	8–10	11	N
217 O.S.	Notaro	+	+	+	+	8–10	11	N

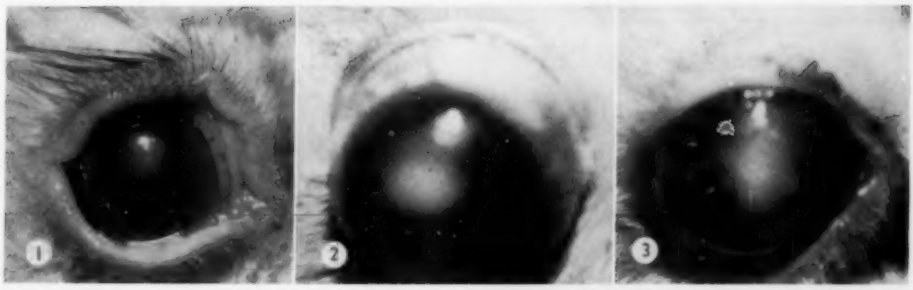
(+) Positive. (0) Negative. (−) Data not available. (N) No exudative reaction seen.
* Rabbit cornea inoculated directly with infective corneal or epithelial scrapings from patients.
† Time of occurrence in days after the inoculation.

in one to two weeks with clear corneas. Of 290 such infections only 24 advanced to a disciform keratitis; this incidence (approximately 8.3 percent) can be compared with a similar low incidence of this disease in human case. In Table 1 are listed the major characteristics seen in this disease in the rabbit. These data suggest that there are at least four features that can be expected to occur in the rabbit in almost every case of disciform keratitis. It must be noted that these characteristics are listed on an absolute plus or minus basis and that no attempt is made to compare the data quantitatively. The last three columns provide available data as to the time when vascularization, opacification, and exudates appeared after the initial virus inoculation.

The finding of a stromal opacity (as determined by slitlamp examination) was positive in every instance. The opacity always extended to some depth in the stroma and is a requisite for disciform keratitis. Corneal thickening was observed 22 out of 24 times and varied in thickness from 50 to 400 percent of the normal cornea depending on the severity of the disease.

Anesthesia was demonstrated in at least 19 of the corneas and was definitely lacking in only one case. The four remaining corneas were not tested for this property. Anesthesia was confined usually to the opacified position of the cornea, but in several instances it was prevalent throughout the whole cornea. The clear portion of the cornea usually remained sensitive or with only partially diminished sensitivity.

Vascularization was demonstrated 20 times and seems to be an almost constant finding in the rabbit cases. This is in contrast to human disciform keratitis in which vascularization is more infrequent and shows

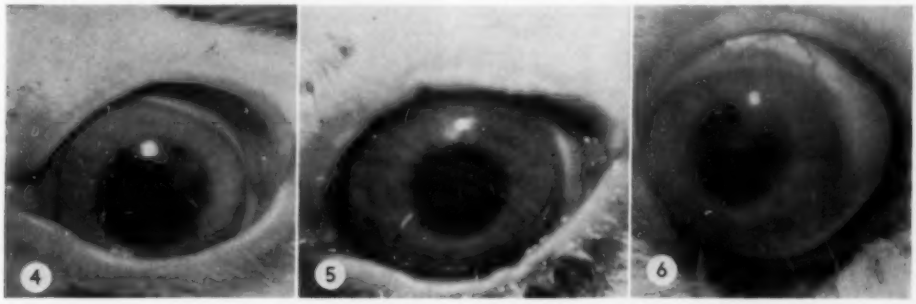


Figs. 1, 2, and 3 (Sery). (Rabbits: 1964, O.D., 17th day; 900, O.D., 14th day; 901, O.D., 14th day, respectively.) Examples of disciform keratitis in the rabbit due to herpes simplex virus infection. These examples are typical of the more pronounced opacities resulting from herpetic ulcers. Note in these cases the generally disc-shaped forms, their approximately central location, and the totality of the opaque tissue surround by a clear zone of apparently normal corneal tissue.

up a month or more after the infection. The early appearance of capillaries in rabbit corneas generally occurred from six to 10 days after inoculation with exceptional cases showing up at extremes of four and 13 days.

The period of onset of corneal opacities was estimated as closely as possible because there was no clear demarcation between the time of the disappearance of epithelial ulcers and the appearance of a stromal opacity. However, corneas were usually hazy or edematic as long as epithelial defects due to dendritic ulcers existed. Some equivocation in time of onset of corneal blood vessels, opacities, and exudates is also apparent in the table due to other problems involving interruption in the observation schedule and daily borderline changes in these features. One feature can be noted in the two columns showing the time of occurrence of corneal vascularity and opacities. This is a tendency for capillary infiltration to either precede or coincide with the occurrence of a stromal opacity. Corneal opacities would make their appearance from seven to 14 days after the inoculation date. Aside from one animal (210) for which data were lacking, no case of corneal opacity occurred later than 14 days.

The formation of a pustular exudate was found to occur quite commonly in the dendritic or disciformlike lesions and usually



Figs. 4, 5, and 6 (Sery). (Rabbits: 948, O.S., 11th day; 939, O.S., 15th day; 922, O.D., 13th day, respectively.) Further examples of herpes simplex induced disciform keratitis but with less pronounced opacities. Two of these examples (948 and 922) are not disc-shaped but nevertheless demonstrated the same characteristics of corneal thickening and stromal involvement for the affected tissue as did the other disciform cases.

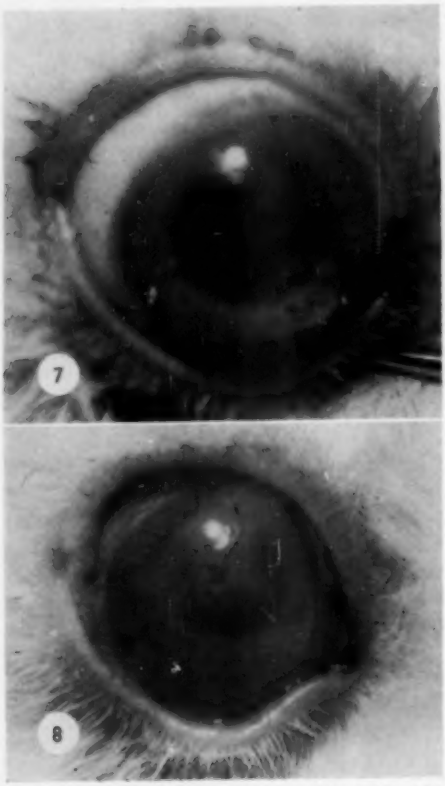


Fig. 7 and 8 (Sery). (Rabbits: 922, O.D., 13th day; 982, O.D., 13th day, respectively). Figure 7 is a duplicate of that shown in Figure 6 but taken after staining the cornea with methylene blue. In this instance the corneal epithelium was not yet healed and a small remnant of the dendritic ulcer still remained. Figure 8 is a better example of a late stage dendritic ulcer around which a disciform (irregular in shape) is beginning to develop. The cornea was stained with methylene blue. Both of these illustrations demonstrate a close association between the sites of persistent dendritic ulcers and the subsequent disciform keratitis lesions that later developed in those corneas.

appeared during the first week of infection. Whenever seen it was cultured and stained for the possible demonstration of secondary bacterial infections, but all attempts to isolate bacteria proved negative. In several instances a small amount of a pustular exudate was inoculated onto normal rabbit eyes with no resultant bacterial infection, but in one instance (939 O.S.) active virus was demonstrated in the pus taken during the dendritic phase of the lesion. The exudate may occur in sparse or large amounts, sometimes causing the lids to become cemented together as it dries exteriorly. This exudate is invariably found to consist primarily of polymorphonuclear leucocytes but also with variable numbers of monoculear leukocytes.

Seven of the disciform cases were observed for extended period of two to six months and, of these, five opacities (862, 894, 900, 901, 309) remained the same or became only partially cleared. The other two (948, 957) lasted two and five weeks respectively during which time the corneas cleared with only slight scar formation on 957. All other animals were killed at varying periods after onset of the disciform lesion from a few days to within a month afterward for purposes of trying to isolate living virus from the total corneal tissue as will be described later. The occurrence of stromal opacities in the rabbit cornea invariably re-

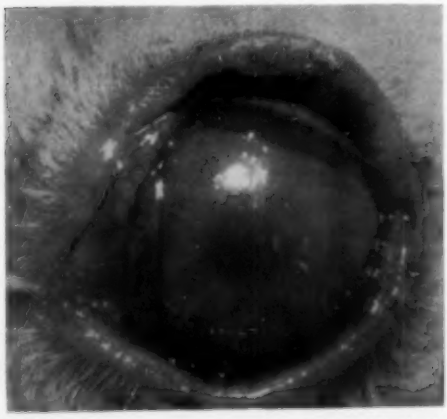


Fig. 9 (Sery). (Rabbit 957, O.S., 11th day). This is an example of an eye that became diffusely opaque, but without losing its transparency, following the disappearance of herpetic dendritic lesions of the epithelium. The stromal lesion resolved after several days into a central opacity resembling a typical disciform keratitis. The above picture demonstrates a roughened surface epithelium which is intact, not taking the usual corneal stains, and was thought to be suggestive of the metaherpetic form of the disease in humans. A heavy vascularization can be seen on the superior limbus.

sulted from the more severe epithelial dendritics and not from mild lesions. Only one instance of a disciform lesion occurred where a concomitant viral count of the inoculum was made and this was in the case of rabbit 988 (O.S.) which received a dose of 30,000 pock-forming units. This eye developed a dendritic ulcer which destroyed most of the corneal epithelium and began to visibly affect the stromal tissue on the ninth day. The contralateral eye of this animal received one-tenth this dose but failed to become even slightly infected. Normally the size of inoculum given for the HF strain of virus consisted of approximately 10^5 to 5×10^6 pock-forming units (the number that usually can be demonstrated in 0.01 ml. of "undiluted" chorioallantoic membrane suspensions). The above rabbit eye therefore represents a disciform lesion that was produced by a lower than average inoculum.

Under slitlamp observation the disciform lesion in its early stages was noted to be opaque in depth, involving the anterior half, or the entire anterior-posterior thickness of the cornea. This portion of the cornea was thickened, sometimes by as much as four times its normal dimension. The opacity extended throughout the stromal fibers appearing as an interlacing network which stands out brightly illuminated against a background of scattered darkened areas. The dark areas were interpreted to be watery pockets in interstitial spaces. Corneal tissue adjacent to the opacity appeared clear. No abrupt increase in corneal thickness was evident in the opaque area as has been described in the human cases of the disease.[2] but in the rabbit there was a gradual thickening of the cornea from the limbus to the opaque area. At a later period the edematous character of the opacity tended to subside and take on a more granular and less filamentous appearance. Disciform opacities which lasted over two months became infiltrated with connective tissue; the over-all corneal thickness at such a time usually remained a little greater than normal. The corneal surface usually became irregular and epithelial facets developed.

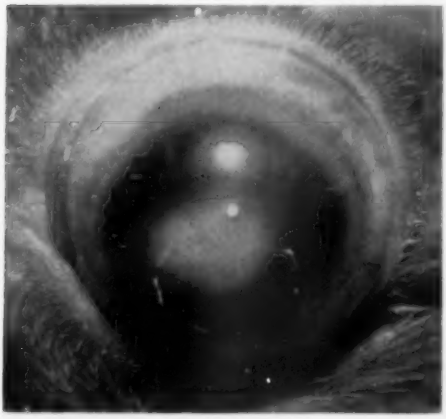


Fig. 10 (Sery). (Rabbit 877, O.S., 86th day). This is an eye with a simulated disciform keratitis, presumably the end-result of a herpes simplex dendritic ulcer, but influenced also by the occurrence of a secondary bacterial infection. The whole cornea became opaque and cleared peripherally very gradually, as seen above, over a period of two months.

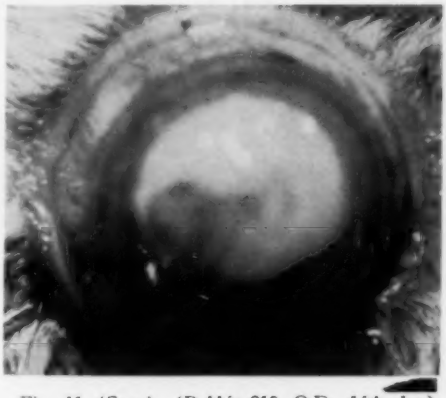


Fig. 11 (Sery). (Rabbit 910, O.D., 14th day). This is an eye with a condition that appeared to be a bacterial corneal ulcer. This cornea did not stain with methylene blue or fluorescein at this stage. A herpetic dendritic keratitis in this eye lasted until the eighth day but became opaque on the ninth day. Bacteria were not isolated or observed by smears at this time but the eye is, nevertheless, believed to be one in which a secondary bacterial infection was involved. Herpes simplex virus was isolated from the total corneal homogenate on the 14th day by passage to another rabbit.

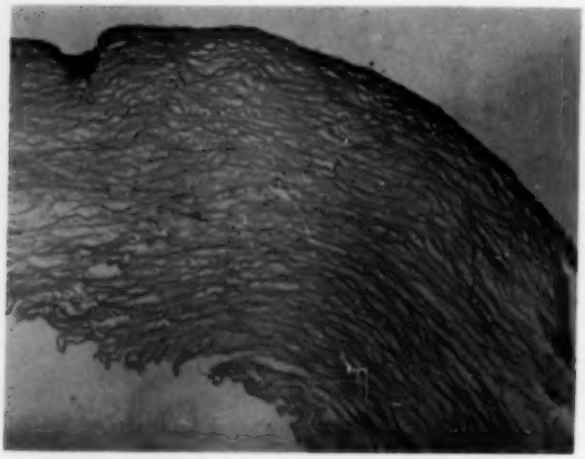


Fig. 12 (Sery). (Rabbit 982, O.S., 14th day). A greatly edematic cornea in a case of disciform keratitis. Cellular infiltration has not yet occurred but usually has by this time. Some intracellular edema is evident in the basal cells of the epithelium.

Under histologic examination the edematic condition of the corneal opacity was verified, Figures 12 and 13. Apparently this was followed at a later stage by cellular infiltrates but the time of appearance of these in different animals did not necessarily follow identical schedules; this may be noted in Figures 12 and 13 in which one animal had edema alone on the 14th day while another had both edema and cellular infiltrates on the 11th day. The cellular reaction varied. In the example of Figure 13 round cells predominated and polymorphonuclear cells were in the minority; in another example, not shown (Rabbit 900, O.D., 148th day), the reverse was true. Figure 14 is an example of a cornea in which an eosinophil-like cell predominated. Further histologic study is needed to determine the pathogenesis of this disease.

Infiltration of capillaries starts roughly from six to 13 days after inoculation, depending probably on the severity and duration of the dendritic ulcer and ordinarily does not occur if the dendritic phase has cleared before the seventh or eighth day. Neovascularization was recorded in two instances on the fourth day and in at least two on the sixth day; these were most likely due to the severity of the herpetic lesions. Neovascularization may involve either the total periphery or only a variable segment of the cornea. In the rabbit corneal vascularization occurs readily and is in direct con-

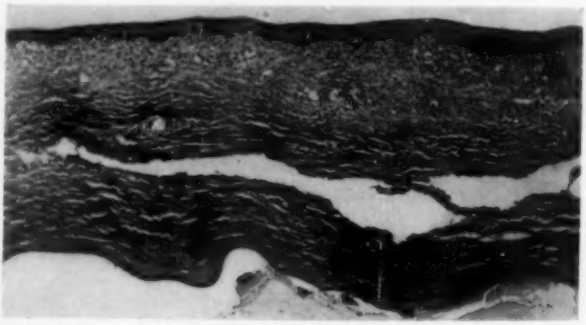


Fig. 13 (Sery). (Rabbit 942, O.S., 11th day). Lamellar disorganization due to edema is present in this cornea but not to as great an extent as seen in Figure 12. The epithelium is thickened irregularly by intracellular edema and general hypertrophy. The stromal reaction involves the anterior half of the cornea and is composed mainly of round cells (mostly lymphocytes) and a few polymorphonuclear leukocytes. A few cells of an equivocal type resembling eosinophils are also present. Connective tissue proliferation is prominent.

trast to human cases where it usually occurs as late as a month or more after the dendritic ulcer and quite often does not develop at all. The corneal vessels seen in rabbit disciform lesions were usually deep with a terminal loop pattern but occasionally superficial vessels also developed. Capillary infiltration of the cornea eventually resulted in either complete, or almost complete, apparent disappearance of vessels.

In a few animals, as for example, 309, O.S., the opacity became almost superficial and thin three months after the original inoculation. In this eye the cornea, under slit-lamp observation, had normal thickness and was clear except for a superficial stromal reflection of light in the pupillary region. One month prior to this the cornea was almost three times normal thickness in the center with a rough and irregular surface, and was edematic and opaque throughout. Vascularization was no longer visible in either instance and central corneal anesthesia was prominent.

Although a severe dendritic keratitis was a common feature preceding a corneal opacity, the latter was not the ultimate result of such infections. On the contrary the general result of even a severe dendritic ulcer was an ultimate return of the cornea to its normal clarity without the production of a disciform opacity. On the other hand an opacity most often developed when a severely infected cornea also become vascularized.

Experiment 2: Attempts to isolate living virus from healed corneas

An attempt was made to demonstrate the presence of virulent or "inactive" virus in rabbit corneas after the primary dendritic keratitis had run through the acute infection and healed. For this purpose 26 corneas with, and 14 without, disciform opacities were selected for testing. The former group included nine corneas, not listed in Table 1, which had suffered from preliminary treatment or secondary bacterial infections during the dendritic infection; other corneas appearing in Table 1 but not in Table 2 were ones that had been formalinized for histologic section. The rabbits were killed at varying intervals following the acute infection and the corneas either immediately tested or preserved in 50-percent glycerine at −20°C. In all of these corneas the epithelium had regenerated as determined by the lack of retention of fluorescein and methylene blue stains. Tests for virulent virus were made by grinding the cornea (after washing in distilled water) in the same manner as described for the chorioallantoic membrane and by suspending the tissue grindings obtained in 0.5 ml. buffer-gelatin solution. After centrifugation at 1,500 rpm for 20 minutes the undiluted supernatant fluid was either inoculated onto normal abraded rabbit corneas or onto the chorioallantoic membrane of 13-day chick embryos for several passages to increase the virus titer and to overcome the presence of antibody formed locally in the cornea. In several instances the final chorioallantoic membrane passage was ground and inoculated onto rabbit corneas.

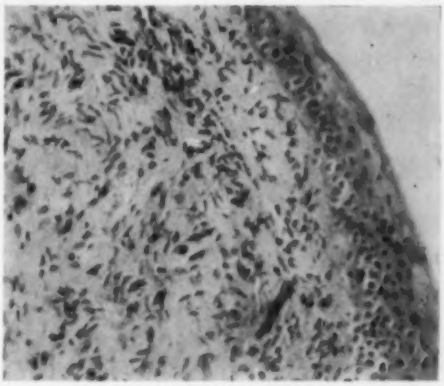


Fig. 14 (Sery). (Rabbit 111, O.D., 13th day). A disciform keratitis complicated by a pyocyaneus bacillus infection resulted in this histologic picture. Extreme connective tissue proliferation and vascularization dominate the tissue reaction. The cellular infiltrate is primarily an eosinophil-like cell. The epithelium is hypertrophic and edematic.

The results of this experiment are summarized in Table 2. Eight corneas from

TABLE 2

RABBIT CORNEAS WITH DISCIFORMLIKE KERATITIS TESTED FOR PRESENCE OF LIVING VIRUS

Group	Rabbit Cornea	Time from Inoculation of Cornea to Day of Death	Attempts at Virus Isolation		
			No. of Serial Passages on Eggs	Direct Passage of Inoculum to Rabbit Corneas	Results
		(days)		(No. inoculated)	
I[1]	862 O.S.	195	4		—[5]
	900 O.D.	148	5		—[5]
	901 O.D.	66		2	—[5]
	922 O.D.	22		2	—[5]
	939 O.S.	23		2	—[5]
	957 O.S.	115	3		—[5]
	999 O.D. O.S.	18	1	2[4]	—[5]
	1964 O.D.	17	5		—[5]
	942 O.S.	11	5		—[6]
	948 O.S.	139	4		—[6]
	971 O.D.	11	1	4[4]	—[7]
	982 O.D. O.S.	14	5		—[7]
	988 O.S.	8	5		—[7]
	1964 O.S.	17	3		—[7]
II[2]	877 O.D. O.S.	86		8	—[5]
	928 O.D.	17		4	—[5]
	990 O.D. O.S.	21	4		—[5]
	992 O.D.	21	1	4[4]	—[5]
	111 O.S.	13	1	2[4]	—[7]
	910 O.D.	15	2	2	—[7 8]
	827 O.D.	174	3	2	—[9]
III[3]	914 O.D. O.S.	41		8	—[5]
	915 O.D. O.S.	23		4	—[5]
	916 O.D. O.S.	11		4	—[5]
	932 O.D. O.S.	150	1	4[4]	—[5]
	945 O.D. O.S.	31	1	4[4]	—[5]
	285 O.D. O.S.		1		—[5]
	286 O.D. O.S.		1		—[5]

[1] Corneas with disciform keratitis.
[2] Corneas with disciform keratitis but which had suffered from other traumatic insults including intracorneal injections of virus or serum, or from secondary bacterial infections.
[3] Control corneas which had recovered from herpes simplex dendritic ulcers and thereafter appeared to be normal and healthy.
[4] Indirect inoculation of corneas from egg passage.
[5] Either egg passage or corneal passage (or both) was negative for virus recovery.
[6] Corneal passage was negative but egg passage was doubtful for virus recovery.
[7] A positive recovery of virus was obtained on eggs and represented a very low count of from 3–13 PFU and only on the first passage.
[8] Represents the only recovery of virus from a rabbit cornea by direct passage to a normal rabbit cornea.
[9] Same results as footnote [7] but with further demonstration of virus up to the third egg passage.

both groups of animals with disciform opacities were tested by direct inoculation into rabbit eyes and of these a single positive dendritic keratitis occurred. This viral isolation from a disciform cornea (No. 910, O.D.) occurred in the group that had had a concomitant bacterial infection superimposed on the viral one. This animal also differed from all others in being the only one that had received preinoculation treatment (that is, 15 days earlier it had received 80 mg. of intramuscular hydrocortisone); whether or not this played a part in the formation of a disciform keratitis or in causing active virus to be present is not known and does not fall within the scope of this paper; the data are included because it represented the only instance of direct cornea to cornea isolation of virus from a postdendritic ulcer in this series, which, as will be shown below, requires a large inoculum in order to produce a detectable infection.

Other positive isolations occurred by cornea to chorioallantoic membrane passage but only very low viral counts were evident. Rabbit corneas from 982, 988, 1964, O.S., 971, O.D., and 111 gave an apparently positive reaction showing typical pock marks on chorioallantoic membranes but ranging only from three to 13 pocks. These five corneas occurred in the two different groups of animals with disciform opacities. Serial passage from chorioallantoic membrane to chorioallantoic membrane failed when tried except for an additional animal (827) where three serial passages of virus on eggs was possible. Two other rabbits, 942 and 948, gave a doubtful virus recovery on eggs where a few atypical pock marks developed. Such marks will often occur on chorioallantoic membranes and it becomes necessary to distinguish them from true, herpes-induced pocks. In these two instances the observed marks were equivocal. No virus recovery was made from any of the control series.

In all but one instance of virus demonstration in disciform corneas the interval between inoculation and death was comparatively small, for example, from eight to 17 days. One possible explanation is based upon the knowledge that local antibody production in the cornea is possible[20] and if present it may neutralize virus when the virus is liberated by grinding the tissue. If intracellular virus has survived in vivo it conceivably would be demonstrated either early in the disease before antibody formation reached an appreciable level or very late when antibody had decreased in concentration.

In an attempt to overcome the activity of local antibody in the cornea an enzyme, papain, was employed to selectively destroy specific virus neutralizing antibody without inactivating living virus that might be present in the corneal stromal cells. Papain was selected because it was shown by Kalmanson, et al.,[21] to be more preferable among the common proteinases for high activity against antibody with low antiphage properties. In this work, controlled tests revealed that concentrations above 0.05 percent were lethal to the virus when incubated for one hour at 37°C. Nevertheless this concentration and incubation period did not materially decrease the antiviral neutralizing capacity of antiherpes simplex rabbit sera and made the system inoperable.

The passage or demonstration of virus by direct cornea to cornea passage is a method that is not as sensitive as the one where eggs are used.[5] A comparison of cornea versus chorioallantoic membrane sensitivity to the virus was made in two groups of rabbits totaling 32 eyes. Tenfold dilutions of virus from 10^{-1} to 10^{-4}, prepared from chorioallantoic membranes as described earlier, were used to inoculate rabbit corneas. Corneas were abraded by central deepithelization or by the cross hatch method and carefully measured amounts of 0.01 to 0.03 ml. of virus dilutions applied. Viral concentrations were computed from pock counts in egg titrations. Results showed a 50 percent infectious dose (ID_{50}) to occur with 1,000 pock-forming units: an ID_{20} with 860 pock-forming units; an ID_{67} with 8,600 to 12900 pock-forming units, and an ID_{100} with 10,000 or more pock-forming units. Observations on these eyes lasted up to 12 days after inoculation and a plus reading was made when one or more definite dendritic marks occurred on the cornea which could be seen with the unaided eye. The ID_{100} which was reached at 10,00 pock-forming units gave a rather mild infection with only about a dozen or less small, well circumscribed dendritics, which cleared after a week. Virus dilutions of 10^{-2} (see Methods) usually represented the ID_{50-100} range. A third group of tests on 24 eyes gave comparable results with regard to dilutions used but the counting of pocks gave poor results due to an unsuitable lot of eggs that was used. Occasionally a very low dose of virus (about 50 to 500 pock-forming units) will produce a dendritic ulcer but these are exceptions and not the rule.

Comment

A basic problem in this study that needs clarification is an understanding of the mechanism that leads to opacification of a fraction of the infected corneas which otherwise would heal with normal transparency. The data of this work do not include many of the negative experiments that were tried in order to induce a disciform opacity at will. Various efforts along this line were tried such as: the use of intracorneal injections of immune serum prior to corneal infections; intracorneal injections of living virus; local treatment of herpetic corneas with prednisolone; intracorneal injections of prednisolone and hydrocortisone before infection; and so forth. More work of this type and along the lines to be discussed below is being furthered in this laboratory and will be reported on at a later date. Although the disciform type of lesion seemed to occur at random in this series, many impressions were obtained in the observation of its "accidental" occurrence as to what may be causing it. Some of the possible factors that may be involved are as follows:

1. Corneal hypersensitivity to herpes simplex virus.
2. Synergistic activity of virus with a secondary bacterial infection.
3. Size of inoculum and severity of infection.
4. The formation of a variant or mutant virus capable of producing an altered pathology.
5. Variation in host response from one individual to another.

In rabbits, onset of the corneal opacity either coincides with, or, quite often, follows the onset of vascularization. This vascularization naturally brings to the cornea an increased supply of plasma proteins and vascular cells which may hinder as well as help in combatting the infection depending upon the immune or hypersensitive state of the individual at the time. One might suggest that if the host has become hypersensitized or allergic to the virus, either indirectly as a result of the corneal infection which extended to other parts of the body, or by infection by some other route, then it could be supposed that further viral insult to the corneal tissue could later manifest itself as a deep tissue reaction corresponding to a so-called disciform keratitis. This idea of a hypersensitive mechanism was suggested by Braley,[4] but adequate experimental evidence for its support is not as yet available. The reason offered for a hypersensitive mechanism is based on the beneficial effects that steroid therapy seemed to offer if given late in the disease when a disciform condition was developing. Thus, it was felt to be analogous with other allergies that benefit from such therapy. One rational basis for support of a hypersensitive mechanism might be postulated as an in vivo passive transfer of an acquired generalized hypersensitivity of the host to its cornea by means of leukocytic infiltration from the limbal and neovascular vessels entering the cornea. The resulting opacity might then follow any contact with virus occurring at the then present or subsequent infections.

In recent years much evidence has been accumulated that fixes the agent or agents responsible for passive transfer of delayed types of hypersensitivity to the mononuclear leukocytes of the sensitive animal (ref. 22-25 and others). Such studies should turn out to be valuable as a new approach to the study of corneal complications of various types, including that of disciform keratitis, in that sensitizing leukocytes may be isolated from systemically treated animals and injected into the cornea of fresh animals with the purpose of demonstrating the phenomenon of hypersensitivity to the particular agent under study. The recent work of Tremaine[25] has demonstrated the value of this type of approach by the passive transfer of sensitivity to horse serum to the rabbit cornea. Comparable studies with herpes simplex virus as well as other agents are currently under way in this laboratory and, it is hoped, may shed more light on the problem of the

mechanisms involved in disciform keratitis.

The suggestion of a hypersensitivity reaction in the cornea is by no means the only hypothesis that may be offered to account for the herpetic induced disciform keratitis. A possibility exists that an associated secondary bacterial infection might work synergistically with the virus leading to a stromal tissue insult. The presence of strong neutrophilic reactions in the eyes of many animals of this series of disciformlike lesions in the early phases of the disease was very suggestive of an associated secondary infection with bacteria even though the culture tests for bacteria proved negative. The absence of positive smear or cultural data on ocular infection is by no means an absolute indication that an associated bacterial or mycotic infection had not been present. If such an infection does occur during an attack of the cornea by herpes virus it could account for the neutrophil tissue reaction. The extension of the corneal pathology into the stromal tissue does not necessarily speak of viral extension per se but might be an effect of a bacterial exo- or endotoxin, or other agent, that is working either secondarily to or synergistically with the superficial viral infection. The almost consistent lack of active virus in the stroma in human or animal cases of disciform keratitis may be real or imagined depending upon whether the virus becomes neutralized by corneal antibody upon being released from its intracellular environment. The damage to stromal tissue that occurs, therefore, does not necessarily require viral particles for its accomplishment, but could be brought about by soluble toxins, antigens, or some other type of microbial product.

A viral toxin could conceivably account for the production of a disciform keratitis. The association of the disciform lesions seen with severe dendritis ulcers suggests this possibility. The implication is that a severe lesion must contain a high concentration of virus with a concomitantly high level of toxin. An alternative argument would be that the virus particle itself acts as a toxic substance and thus causes stromal tissue damage. One argument disfavoring this hypothesis is that many severe dendritic ulcers are seen in rabbits which heal spontaneously with no stromal complications.

The possibility that a mutant virus might be a factor leading to pathologic changes in the stromal tissue was considered in this work. Study of all data, however, did not lend favorable support to this idea due to the fact that eye to eye passages from disciform animal cases did not necessarily produce a disciform keratitis. If, however, a mutant virus did appear late in the dendritic phase of the infection, when virus passage attempts usually fail, it would not be detected and would either die out or produce only minor corneal infections. Rabbit 999, O.D., is one example that argues against a mutant virus. Both eyes of this animal were inoculated simultaneously by passing small pieces of infected epithelium from a stock culture (Rabbit 994). There was no quantitation of virus inoculum in this instance and therefore both eyes cannot be considered to be equally treated. Nevertheless, the severity of the corneal reaction to the virus was comparable during the first few days. The dendritic ulcer of the O.S., however, started to diminish after five days and never became vascularized while that of the O.D. started to diminish after seven days at which time capillary infiltration was first noticed on the superior limbus. After 10 days the capillaries were well extended into the stromal tissue two to four mm. from the limbus and were in contact with a disciform opacity that was now developing. If a mutant virus was involved it presumably would have affected both corneas in the same manner unless the disciform pathology of the O.D. was the result of a single mutant particle in the inoculum.

Katznelson[26] reported cases of disciform herpetic keratitis due to thiamine insufficiency, and Hartmann[27] had cases of disciform keratitis as a result of exposure to

hydrogen sulfide gas. The occurrence of the disease under such circumstances suggests a possible metabolic disturbance that may be basically involved in the condition as brought about by infectious agents. The work of Katznelson might suggest that a vitamin deficiency in corneal viral infections is the mechanism that triggers the onset of the disciform opacity. Further study into these areas should be made and could prove to be very illuminating.

Summary

1. Spontaneous occurrence of corneal opacities or "disciform keratitis" in rabbits following herpes simplex dendritic ulcers have been described. A total of 24 such cases out of 290 infected eyes were studied.

2. In rabbits the most common characteristics included an active, stromal inflammation, with corneal thickening due to edema, neovascularization, anesthesia—usually confined to the opacity, and, quite often, cellular infiltrates of polymorphonuclear and mononuclear cells, which in many cases also appeared as an exudate on the surface. Opacities, at first primarily edematic, later assumed a more granular character. Opacities were mostly central and varied in shape from discoid to irregular types. Stromal lesions were long lasting and often permanent when severe, but were otherwise transient, leaving a clear and normal appearing cornea after two to eight weeks.

3. Very few viral isolates were obtained from these corneas after the disappearance of the dendritic ulcer and the establishment of the disciform lesion. In these few positive demonstrations the virus content was low, or represented a period when the virus was either inactive or had become neutralized by corneal antibody during technical manipulation.

4. The 50-percent minimal infectious dose of herpes simplex on the rabbit cornea was found to require above 1,000 pock-forming units—a factor which must be considered when one is trying to isolate virus from tissues by inoculating rabbit corneas.

5. Various possible hypotheses for the mechanism of corneal opacification were discussed.

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ELABORATION OF BICARBONATE ION IN INTRAOCULAR FLUIDS*

VII. Studies with $NaHC^{14}O_3$ on the effect of diamox on rabbit aqueous humor

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In the previous paper[1] were reported the results of a study of the kinetics of the bicarbonate ion in the aqueous humors of the rabbit eye following the intravenous injection of C-14 labeled $NaHCO_3$. On the basis of the available evidence and the results of the C-14 studies it was proposed that the bicarbonate ion in the aqueous humor of the posterior chamber arises chiefly from the bicarbonate ion in the plasma and that this is the major chemical species in which form the substance is transported across the blood-aqueous barriers.

In view of the apparently high permittivity of the blood-aqueous barriers to the bicarbonate ion it was further proposed that part of the bicarbonate ion in the aqueous humors is "carried" or "draggged" in with the bulk or unidirectional flow of fluid into the eye. This route together with the presumed active transport of the bicarbonate ion into the posterior chamber are considered to describe the two main pathways of entrance of the bicarbonate ion into the aqueous humor of the rabbit eye.

This report is a study of the effect of an intravenous injection of acetazolamide (Diamox) upon the kinetics of the bicar-

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bonate ion in the aqueous humors of the rabbit eye studied with the use of intravenously injected C-14 labeled $NaHCO_3$.

Experimental

Except for the administration of acetazolamide (Diamox*), 30 mg./kg. body weight, as a solution of the sodium salt (30 mg./ml.) the entire experimental procedure was the same as described in the previous paper. The drug was injected intravenously 45 minutes prior to the administration of the radioactive $NaHCO_3$, and the aqueous humors removed from the first eye five to 10 minutes later and from the other eye 10 to 20 minutes later. These time periods were chosen to coincide with the time required (about 60 minutes) for the bicarbonate ion concentration in the aqueous humor of the posterior chamber to fall maximally (21.6 percent) following the injection of Diamox. The corresponding fall in the anterior chamber fluid after this period of time was about 12 percent.[2]

Experiments designed to determine the effect of Diamox acting over shorter periods of time were also performed. For this purpose the labeled $NaHCO_3$ was injected immediately following the injection of the Diamox. The aqueous humors were removed five to 15 minutes after the injection of the $NaHCO_3$. In all experiments samples of arterial blood plasma were prepared from whole blood removed at appropriate time periods.

Results

A positive correction factor of 1.228 was applied to the measured radioactivity of the plasma samples, as previously determined. No correction factor was required for the aqueous humor samples.[1]

In an attempt to minimize the variability from animal to animal of the levels of radioactivity and the rates of decrease in both plasma and posterior chamber fluid, previously observed in untreated animals, some experiments with Diamox were performed on litter-mate rabbits whose weights were approximately the same within 0.5 pounds. These results indicated the same degree of variability as observed with rabbits chosen at random without regard to litter-mate source.

In all cases the time periods indicated refer to the time after the administration of the $NaHC^{14}O_3$.

C-14 analyses of plasma and aqueous humors

($NaHC^{14}O_3$ injected 45 minutes after the injection of Diamox—Table 1).

Plasma. The results of 12 experiments are given in Table 1.† From a semilog plot of the activity of plasma samples against time it was apparent that the radioactivity disappeared at a single exponential rate. Accordingly, the best straight line was drawn from the plasma data (a minimum of three points) on a semilog plot by graphic methods which meet the criteria of the method of least squares. A slope of each straight line represented the rate of disappearance of C-14 in the plasma in terms of percent of total C-14/min. and the values are listed under the column percent $\triangle$/min. While the individual values varied from animal to animal, the average rate of disappearance in the 12 experiments was 5.65 percent/min. This corresponds to an average time for 50 percent disappearance of radioactivity of 12.27 minutes. The time interval during which samples of whole blood was removed from the ear artery for the preparation of plasma was from five to 30 minutes, and usually with not less than eight to 10 minutes between taps.

Posterior chamber. The radioactivity of the aqueous humor of the posterior chamber is recorded in Table 1, as counts/min./0.2 ml. in the columns headed CPM (t_1) and CPM(t_2); the figures in parentheses refer

* Diamox was generously supplied by the Lederle Laboratories Division of the American Cyanamid Corporation, Pearl River, New York.

† The following abbreviations have been used in the presentation and discussion of results: Pl, arterial blood plasma; A. C., anterior chamber; P. C., posterior chamber; CPM, counts per minute per 0.2 ml. of sample.

TABLE 1

C^{14} ANALYSES OF PLASMA AND AQUEOUS HUMORS OF ANTERIOR AND POSTERIOR CHAMBER OF RABBITS FOLLOWING INTRAVENOUS INJECTION OF $NaHC^{14}O_3$ 45 MINUTES AFTER INTRAVENOUS INJECTION OF DIAMOX (30 MG./KG.)

Plasma		Posterior Chamber Fluid					Anterior Chamber Fluid				PC/AC	
No.	% Δ/min.*	CPM (t_1)	PC/Pl	CPM(t_2)	PC/Pl	% Δ/min.*	CPM(t_1)	AC/Pl	CPM(t_2)	AC/Pl	t_1	t_2
1	5.47	12,300 (4.75)	1.66	6,270 (16)	1.57	5.97	6,305 (5)	0.86	5,781 (17)	1.52	1.95	1.09
2	6.44	7,512 (6.75)	1.30	5,440 (13.75)	1.47	4.60	4,640 (7)	0.83	3,913 (14)	1.09	1.62	1.39
3	3.99	11,126 (8.25)	2.23	5,968 (16.3)	1.66	7.52	4,108 (8.5)	0.82	4,351 (16.81)	1.24	2.71	1.37
4	5.23	15,053 (7.5)	2.32	8,629 (15)	1.96	7.39	5,042 (7.5)	0.78	5,116 (15.5)	1.19	2.99	1.69
5	7.37	10,526 (7.25)	1.85	7,834 (15)	2.44	3.78	4,869 (8.5)	0.92	4,842 (15.25)	1.42	2.16	1.62
6	10.90	16,746 (7.5)	1.50	10,913 (15.25)	2.27	5.49	5,977 (8.0)	0.56	6,351 (15.5)	1.35	2.80	1.72
7	6.77	13,972 (7.75)	2.12	8,210 (15.5)	2.11	7.32	5,963 (8.25)	0.93	5,204 (16.25)	1.41	2.34	1.58
8	5.01	16,906 (7.75)	2.64	7,531 (16.75)	1.84	8.96	6,912 (8.3)	1.08	6,070 (17.5)	1.56	2.45	1.24
9	4.33	11,506 (9.0)	1.51	8,418 (17.25)	1.59	3.82	4,397 (9.5)	0.60	4,753 (17.5)	0.91	2.62	1.77
10	4.60	8,181 (9.0)	1.78	6,344 (18.25)	2.11	2.76	3,107 (9.25)	0.68	3,179 (18.75)	0.80	2.63	2.00
11	3.77	9,572 (7.25)	1.62	7,920 (15.0)	1.80	2.46	2,689 (8.0)	0.47	4,111 (15.25)	0.93	3.56	1.93
12	3.94	6,550 (7.5)	1.72	4,876 (4.75)	1.71	4.04	2,687 (8.0)	0.73	1,970 (15.0)	0.70	2.44	2.48
Av.	5.65		1.85		1.88	5.34		0.77		1.18		
S.D.†	1.94		0.377		0.290	2.00		0.166		0.288		
S.E.M.‡	0.56		0.109		0.084	0.58		0.048		0.087		

* % Δ/min.: Percentage of the radioactivity decreasing per minute.
† S.D.: standard deviation.
‡ S.E.M.: standard error of the mean.

to the time of withdrawal of the fluid. The variable levels of activity observed in each of the two columns are essentially a reflection of the variability of the levels of activity in the plasma and of the different times of withdrawal of the fluid. More informative and consistent data are obtainable from the ratio of the levels in the posterior chamber to those in the plasma (PC/Pl), for corresponding periods of time. The plasma values for the given experiment were read from the best straight lines of the individual semi-log plots. The average values for the PC/Pl ratios at t_1 and t_2 were 1.85 and 1.88, respectively, and statistically not different from each other. This suggested that the relationship of the activity in the posterior chamber to that in the plasma was essentially constant, at least during the indicated interval of time (five to 20 min.).

In order to define more precisely the actual relationship between the activities of these two fluids, the individual PC/Pl ratios were plotted against time, as in Figure 1-A. Despite the wide scatter of the experimental points it was obvious that the trend was best described by a straight line between the two series of points. Further resolution was accomplished by plotting the calculated averages of values associated with the relatively more dense time groupings, such as between five to 9.5 minutes and 15 to 19.5 minutes. The results of such calculations are given in Table 2 with the points plotted in Figure 1-B. It is obvious that the two points form a straight line (average 1.883) parallel with the time co-ordinate of the plot.

With the establishment of the constancy of the PC/Pl ratio with time, it was at once apparent that the radioactivity of the aqueous humor in the posterior chamber must have disappeared at about the same rate as did that in the plasma. That this was the case was confirmed by comparing the averages of the individual rates of disappearance (percent △/min. in table 1). The results show a value of 5.34 percent/min. for the posterior chamber fluid which was, indeed, not significantly different ($P > 0.60$) from 5.65 percent/min. for the plasma.

Anterior chamber. The radioactivity of the aqueous humor of the anterior chamber is recorded in Table 1, as counts/min./0.2 ml. in the columns headed CPM(t_1) and CPM(t_2); the figures in parentheses refer to the time of withdrawal of the fluid. As with the data of the posterior chamber fluid, here too the variability of the levels of activity observed in both columns under anterior chamber fluid was essentially a re-

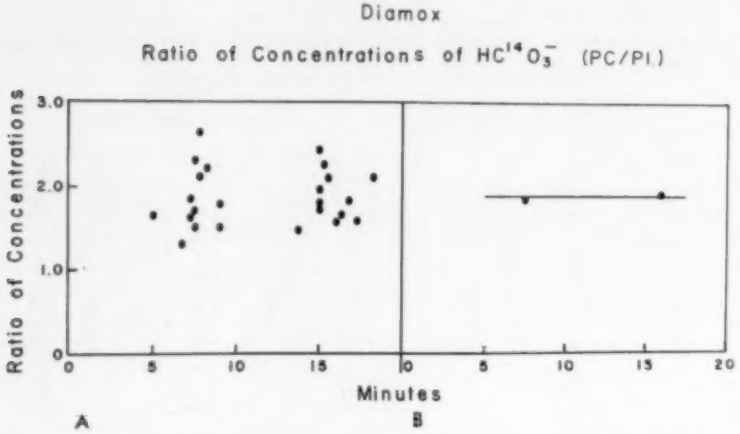

Fig. 1 (Green and Sawyer). (A) Plot of all individual radioactivity concentration ratios (PC/Pl) given in Table 1. (B) Plot of the average of the individual radioactivity concentration ratios (PC/Pl) grouped according to time (table 2).

flection of the variability of the levels of activity in the plasma from animal to animal and of the different times of withdrawal of the fluid. In order to minimize the influence of the individual variability and to achieve more informative and consistent data, the ratios of the activity in the anterior chamber fluid to that in the plasma were calculated (AC/Pl). It can be seen that in every case but one (experiment 12) the ratio of AC/Pl was higher for the later time period. The over-all averages were 0.77 and 1.18 for t_1 and t_2, respectively.

In order to define more precisely the actual time relationship between the activity in the aqueous humor of the anterior chamber and that in the plasma the individual AC/Pl values were plotted against time, as in Figure 2-A. Despite the relatively wide scatter of the points there is a noticeable tendency for the ratio to rise. Further definition of the trend of the points was achieved by plotting the calculated averages of the values associated with the relatively more dense time groupings, such as between five to 9.5 minutes and 10 to 14.5 minutes. Results of such calculations are given in Table 3 with the points plotted in Figure 2-B. It is obvious that the ratio rose during the five-to-20-minute interval of time. Actually the curve should not start at the origin. However, because of the relatively slow rise of radio-

TABLE 2

RATIO OF CONCENTRATIONS OF RADIOACTIVITY (PC/Pl) GROUPED ACCORDING TO TIME

Time Minutes	No. of Expts.	Average	S.D.*	S.E.M.†	Av. Time Minutes
5– 9.5	12	1.854	0.378	0.109	7.54
10–14.5	1				
15–19.5	11	1.915	0.275	0.0837	15.94
20–25	0				
Weighted Average		1.883			

* S.D.: standard deviation.
† S.E.M.: standard error of the mean.

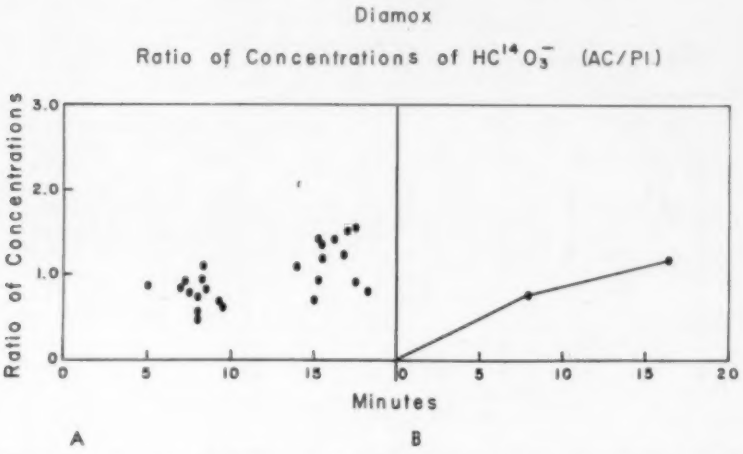

Fig. 2 (Green and Sawyer). (A) Plot of all individual radioactivity concentration ratios (AC/Pl) given in Table 1. (B) Plot of the average of the individual radioactivity concentration ratios (AC/Pl) grouped according to time (table 3).

activity in the anterior chamber compared to the very rapid and immediate rise in the plasma, for all practical considerations the curve may be considered to start at the origin.

Relationship of radioactivity in aqueous humors of posterior and anterior chambers. The ratios of the activity of the aqueous humor of the posterior chamber to that of the anterior chamber for both eyes of each rabbit are given in Table 1 in the last two columns. The t_1 and t_2 refer to the time when each eye was tapped. Since the anterior chamber fluid was removed usually within one minute of the time after the posterior chamber fluid was removed, for the sake of simplicity, the t_1 and t_2 refer to the time of the posterior chamber tap. The individual values are plotted against time in Figure 3-A. Although it is quite apparent that the ratio tended to decrease with time, better definition of the relationship between PC and AC activities was obtained from the averages of the values grouped according to time intervals, such as for five to 9.5 minutes and 10 to 14.5 minutes (table 4). A plot of these average values against the average time of the interval gave, as shown in Figure 3-B, a distinctly decreasing trend.

C-14 ANALYSES OF PLASMA AND AQUEOUS HUMORS

($NaHC^{14}O_3$ injected immediately after the injection of Diamox—Table 5).

The results of six experiments are given in Table 5. Because of the close similarity

TABLE 3

RATIO OF CONCENTRATIONS OF RADIOACTIVITY (AC/PL) GROUPED ACCORDING TO TIME

Time Minutes	No. of Expts.	Average	S.D.*	S.E.M.†	Av. Time Minutes
5– 9.5	12	0.77	0.166	0.0480	7.88
10–14.5	1				
15–19.5	11	1.185	0.288	0.0867	16.39
20–25	1				

* S.D.: standard deviation.
† S.E.M.: standard error of the mean.

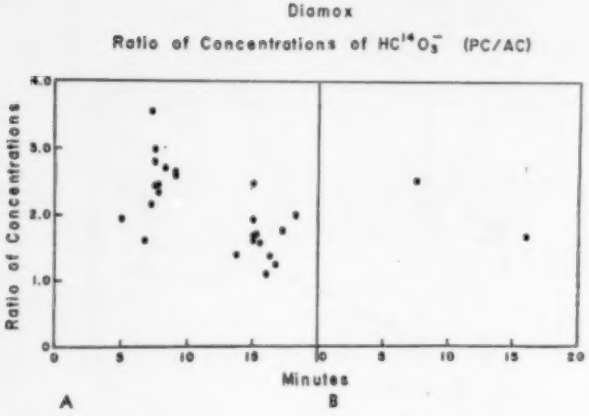

Fig. 3 (Green and Sawyer). (A) Plot of the individual radioactivity concentration ratios (PC/AC) given in Table 1. The time of removal of the anterior chamber fluid was taken as the same as that of the PC fluid. (B) Plot of the average of the individual radioactivity concentration ratios PC/AC) grouped according to time (table 4).

of the individual experimental time periods in each group, t_1 and t_2, the average values may be compared directly. Thus, the PC/Pl ratio of concentrations of activity at the time of withdrawal of the aqueous humor (average t_1, 5.70 minutes) was 1.560 while that at t_2 (average, 11.75 minutes) was 1.651. The corresponding values of the AC/Pl ratios were 0.537 at an average t_1 of 6.30 minutes, and 0.756 at an average t_2 of 12.10 minutes. The PC/AC ratio of concentrations at the t_1 and t_2 corresponding to the times of removal of the PC sample, were 3.02 and 2.26, respectively.

Discussion

The rabbits used in this study were considered to have been normal and representative of adult pigmented rabbits and, as such, were assumed to have physiologic and biochemical properties similar to those of the animals used in the previous study[1] without Diamox.

In evaluating the effects of Diamox it is necessary to recall that in the previous study the dynamics of the bicarbonate ion was followed during the normal steady-state conditions of untreated animals. Under the conditions of the present study, that is, 50 to 60 minutes after the injection of Diamox, it had been found[2] that while the bicarbonate ion concentration of the arterial plasma of rabbits remained essentially unchanged, that in the aqueous humor of the posterior chamber decreased 21 percent and that in the aqueous humor of the anterior chamber decreased 12 percent.

Comparison of the kinetics of the bicarbonate ion

As studied with C-14 labeled $NaHCO_3$, with and without the injection of Diamox (45 minutes prior to that of $NaHC^{14}{}_3$).

Rates of decrease of radioactivity in plasma. Comparison of the average rates of decrease of the radioactivity concentration

TABLE 4

Ratio of concentrations of radioactivity (PC/AC) grouped according to time

Time Minutes	No. of Expts.	Average	S.D.*	S.E.M.†	Av. Time Minutes
5– 9.5	12	2.52	0.478	0.138	7.54
10–14.5	1				
15–19.5	11	1.68	0.365	0.110	15.94
20–25	0				

* S.D.: standard deviation.
† S.E.M.: standard error of the mean.

TABLE 5

C^{14} ANALYSES OF PLASMA AND AQUEOUS HUMORS OF ANTERIOR AND POSTERIOR CHAMBERS OF RABBITS FOLLOWING INTRAVENOUS INJECTION OF $NaHC^{14}O_3$ IMMEDIATELY AFTER THE INTRAVENOUS INJECTION OF DIAMOX (30 MG./KG.)

Plasma		Posterior Chamber Fluid					Anterior Chamber Fluid				PC/AC	
No.	% Δ/min.*	CPM(t_1)	PC/Pl†	CPM(t_2)	PC/Pl†	% Δ/min.*	CPM(t_1)	AC/Pl‡	CPM(t_2)	AC/Pl†	t_1	t_2
1	4.63	4,169 (5.5)	1.522	3,562 (10.5)	1.612	3.14	1,276 (6.0)	0.473	1,324 (10.5)	0.602	3.27	2.69
2	5.86	4,927 (5.0)	1.564	3,888 (10.5)	1.698	4.35	1,474 (6.0)	0.477	1,457 (10.75)	0.650	3.34	2.67
3	5.25	5,619 (5.25)	1.488	3,977 (15.25)	1.768	3.44	1,954 (6.0)	0.528	1,758 (15.75)	0.803	2.88	2.26
4	7.59	8,775 (5.25)	1.688	5,437 (13.25)	1.942	5.96	2,431 (5.75)	0.486	2,431 (13.75)	0.900	3.61	2.24
5	3.78	5,606 (6.5)	1.900	4,488 (10.75)	1.753	5.94	1,891 (7.0)	0.654	2,060 (10.75)	0.824	2.96	2.18
6	3.92	4,334 (6.5)	1.235	3,395 (10.25)	1.132	5.81	2,091 (7.0)	0.603	2,260 (11.0)	0.756	2.07	1.50
Av.‡	5.175		1.560		1.651	4.773		0.537		0.756	3.02	2.26
S.D.	1.285		0.205		0.252	1.189		0.069		0.102		
S.E.M.	0.524		0.084		0.103	0.485		0.028		0.042		

Average time (minutes): PC(t_1), 5.70; PC(t_2), 11.75; AC(t_1), 6.30; AC(t_2), 12.10.

* % Δ/min.: Percentage of radioactivity decreased/minute.
† Ratio of concentrations of activity at time of withdrawal of aqueous humor.
‡ Average; S.D.: standard deviation; S.E.M.: standard error of mean.

in the plasma of normal and Diamox treated rabbits (table 3, previous paper, and table 1, present paper) indicates that the drug had no significant effect upon this system, even within 50 percent confidence limits (normal, 6.22 percent/min. and with Diamox, 5.65 percent/min.). This is in agreement with the findings of Green and co-workers[2] and of Becker[3] that the HCO_3^- concentration in the plasma was not appreciably affected by the intravenous injection of Diamox in 60 minutes, but not in accord with the findings of Langham and Lee[4] that Diamox lowered the HCO_3^- concentration of the plasma during the same time period about 25 percent.

Rates of decrease of radioactivity in aqueous humor of posterior chamber. Comparison of the average rates of decrease of the radioactivity concentration in posterior chamber fluid of normal and Diamox-treated rabbits (table 3, previous paper, and table 1, present paper) indicates that the drug had no significant effect upon this system, even within 50 percent confidence limits (normal, 5.66 percent/min. and with Diamox, 5.34 percent/min., a relative difference of only six percent). In view of the similarity of the rates of decrease of radioactivity in the corresponding plasmas, this similarity in the rates of decrease of radioactivity in the posterior chamber suggests that the fundamental dependence of the rate of change of radioactivity in the posterior chamber upon the corresponding rate of change in the plasma[1] apparently has not been affected by 50 to 60 minutes of Diamox action.

Ratios of concentrations of radioactivity—PC/Pl. Comparison of the average ratios,

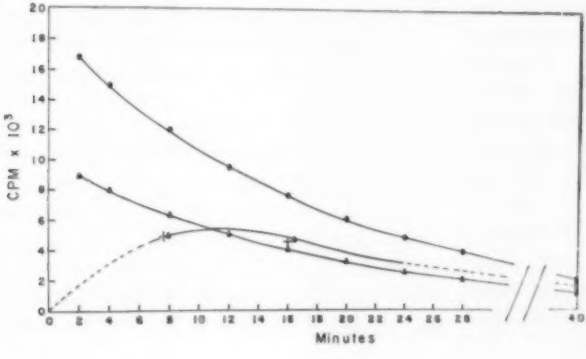

Fig. 4 (Green and Sawyer). Radioactivity concentration of aqueous humors and plasma versus time. The radioactivity concentration is given in terms of counts/min./0.2 ml. All curves are averages as explained in the text under "Discussion." Plasma curve, —▲—▲—; PC curve, —●—●— (calculated from average PC/Pl of 1.883); AC curve, —Δ—Δ— (calculated from AC/Pl values grouped according to time); and —+—+ (calculated from PC/AC values grouped according to time).

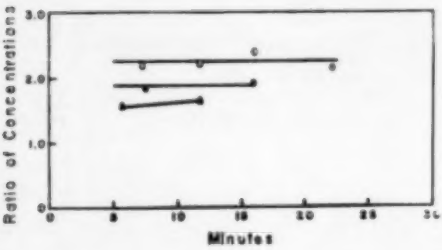

Fig. 5 (Green and Sawyer). Comparison of average PC/Pl ratios of concentration of radioactivity: normal (—0—0—); Diamox injected 45 minutes previously (—●—●—); Diamox injected at zero minutes (—▲—▲—). The samples of aqueous humor were drawn at the indicated time periods (averages) after the administration of $NaHC^{14}O_3$; the plasma values at the same time periods were averages of the individual semi-logarithmic plots of radioactivity concentrations versus time.

for the period of the experiments, of the concentrations of radioactivity of posterior chamber fluid to that of the plasma (table 4, fig. 1-A and 1-B, previous paper, and table 2, fig. 1-A, 1-B, present paper) indicates that the PC/Pl ratio in the Diamox-treated animals was 17 percent less than that in the normal animals (1.883 and 2.259, respectively). This is graphically illustrated in Figure 5. The difference was significant within better than 99 percent confidence limits ($P < 0.01$). Since 50 to 60 minutes of Diamox action did not significantly affect the rate of decrease of the concentration of radioactive bicarbonate ion in the plasma or the bicarbonate ion concentration itself,[2,3] then a comparison of the PC/Pl ratios of radioactive bicarbonate ion concentrations, before and after the injection of the drug, is basically a measure of the effect of Diamox upon the *concentration* of radioactive bicarbonate ion in the aqueous humor of the posterior chamber. Thus the observed decrease of 17 percent in the PC/Pl ratio of radioactive concentrations of bicarbonate ion may be taken as a 17 percent decrease in the concentration of radioactive bicarbonate ion in the posterior chamber fluid. This is in good agreement with the non-radioactive chemical findings, already referred to,[2] that during the same time period under consideration the intravenous injection of Diamox decreased the bicarbonate ion concentration of the posterior chamber fluid by about 21 percent.

Ratios of concentrations of radioactivity —AC/Pl. Comparison of the average ratios, for the corresponding grouped time periods, of the concentrations of radioactivity in the anterior chamber fluid to that of the plasma (table 5, fig. 2-A, 2-B, previous paper, and table 3, fig. 2-A, 2-B, present paper) indicates that the AC/Pl ratio in the Diamox-treated animal was 30 percent lower for the five-to-9.5-minute period and 36 percent lower for the 15- to 19.5-minute period than the corresponding AC/Pl ratios in the normal animal (0.77 and 1.00, respectively, for the five- to 9.5-minute period and 1.185 and 1.629, respectively, for the 15- to 19.5-minute period). This is graphically illustrated in Figure 6. The differences were significant within 95 percent confidence limits.

For reasons similar to those discussed in the preceding section, a comparison of the AC/Pl ratios of radioactive bicarbonate ion concentration, before and after the injection of the drug, should provide an estimate of the effect of Diamox upon the *concentration* of radioactive bicarbonate ion in the anterior chamber fluid. Thus the observed decrease of some 30 percent in the AC/Pl ratio of radioactivity should indicate a 30 percent decrease in the concentration of radioactive bicarbonate ion in the anterior chamber fluid. This is *not* in good agreement with the non-radioactive chemical findings already referred to,[2] that during the same time period Diamox caused a 12 percent decrease in the bicarbonate ion concentration. The reason for this discrepancy will be discussed later.

Ratios of concentrations of radioactivity —PC/AC. Comparison of the average ratios, for the corresponding grouped time periods, of the concentrations of radioactivity in the aqueous humor of the posterior chamber to that of the anterior cham-

ber (table 6, fig. 3-A, 3-B, previous paper, and table 4, fig. 3-A, 3-B, present paper) indicates no appreciable difference between the two ratios. However, before concluding that Diamox had no effect upon the PC/AC ratio of radioactivities, it should be observed, from previous studies, that the corresponding ratios of the bicarbonate ion concentrations in the two chamber fluids was decreased only about 10 per cent in one hour following the injection of Diamox.[2] Such a magnitude of change in the radioactivity experiments would be difficult to detect for this ratio, in view of the relatively wide scatter of the data. In addition, because of insufficient number of grouped timed periods available in the present study, comparison of the rates of changes of the PC/AC ratios of radioactivities was not possible.

Average radioactivity concentration-time curves of aqueous humors and plasma (fig. 4, previous paper, and fig. 4, present paper). Inspection of the two sets of curves describing the average rates of change of radioactivity concentrations for the three body fluids with and without pretreatment of the animal with Diamox reveals an essential similarity in form and shape for the corresponding curves. The difference between the corresponding PC/Pl and AC/Pl ratios has already been discussed.

While the radioactivity concentration relationships between the three body fluids of the untreated normal animals may be extrapolated to infinite time,[1] the corresponding relationships in the Diamox-treated animals may not be safely extrapolated, because of the differences in changes in bicarbonate ion concentrations in the three body fluids with longer time periods.[2]

Mode of action of Diamox

According to the secretion-diffusion theory of aqueous humor formation[5,6] bicarbonate ions are presumed to be formed in the epithelium of the ciliary body according to the reaction, $HO^- + CO_2 \rightleftharpoons HCO_3^-$. The HO are considered to arise from the oxidation of

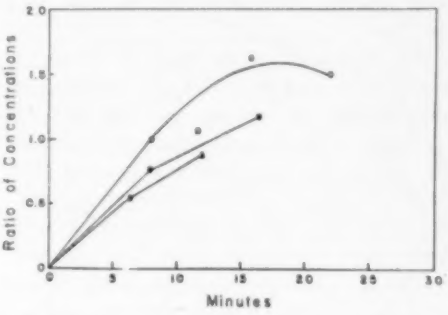

Fig. 6 (Green and Sawyer). Comparison of average AC/Pl ratios of concentrations of radioactivity: Same legend and conditions as in Figure 5.

reduced cytochrome oxidase in the epithelium while the CO_2 is postulated as being derived from the HCO_3^- of the blood in the stromal capillaries. The bicarbonate ions thus formed are said to diffuse into the posterior chamber. This sequence of events comprises the fundamental basis for the maintenance of a higher concentration of bicarbonate ions in the aqueous humor than in the circulating arterial plasma. This excess bicarbonate ion concentration has been suggested as being chiefly responsible for the measured hypertonicity of the aqueous humor of the rabbit compared to the plasma. As a direct consequence of this osmotic gradient a continuous unidirectional flow of fluid from the plasma into the posterior chamber is presumably maintained. Although the validity of Friedenwald's formulation has been questioned on physical chemical as well as biochemical grounds,[7] and his use of vital dyes as a basis for the drawing of conclusions as to the physiologic properties of tissue has been criticized,[8]* nevertheless, the concept that the excess bicarbonate ion in the aqueous humor of the rabbit eye plays the pivotal role in the formation of the aqueous humor has been

* In addition it should be mentioned that in view of the penetration of anions as well as cations from the plasma into the posterior chamber, Friedenwald's basis for postulating an irreciprocal permeability of the ciliary epithelium would appear not to be justified.

most widely accepted during the past two decades.

Friedenwald,[9] on the basis of Becker's[3] initial studies on the effect of Diamox upon the bicarbonate ion concentration and the dynamics of the aqueous humor formation in the rabbit eye, incorporated a vital role for carbonic anhydrase in his previously postulated series of reactions for the formation of bicarbonate ions by the ciliary body. According to this carbonic anhydrase in the ciliary epithelium serves fundamentally to catalyze the hydration of CO_2, presumably from the blood bicarbonate, according to reaction (1):

$$CO_2+H_2O \rightleftharpoons H_2CO_3 \qquad (1)$$

In this way carbonic acid, H_2CO_3, is made readily available to neutralize the hydroxyl ions, presumably forming in the epithelium, to produce bicarbonate ions, according to reaction (2):

(2) $$H_2CO_3+OH^- \rightleftharpoons HCO_3^- + H_2O \qquad (2)$$

In the stroma, on the other hand, carbonic anhydrase is postulated to catalyze the dehydration of carbonic acid (reaction [4]) arising from the neutralization of plasma bicarbonate by the H^+ (reaction [3]) generated by dehydrogenase reactions in the stroma.

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \qquad (3)$$

$$H_2CO_3 \rightleftharpoons H_2O + CO_2 \qquad (4)$$

In other words, according to Friedenwald, carbonic anhydrase fundamentally serves to provide the buffering capacity of the ciliary body, within the context of the postulated redox reactions.[9] Therefore, inhibition of the carbonic anhydrase catalyzed reactions (1) and (4) would lead to an accumulation of unneutralized OH^- in the epithelium or H^+ in the stroma, depending upon the site of action of the drug. In any event the resulting pH change (increase in the epithelium or decrease in the stroma) would be large enough to inhibit the redox reactions and result in a decreased rate of elaboration of bicarbonate ions and of secretion of aqueous humor.

Starting with the basic formulations of the secretion-diffusion theory of aqueous humor formation and the associated fundamental role of carbonic anhydrase postulated by Friedenwald, it would seem that elucidation of the mode of action of Diamox upon the rabbit eye depends significantly upon information regarding the following five considerations:

1. Source of the bicarbonate ion in the aqueous humor.

2. Role of carbonic anhydrase in the elaboration of the bicarbonate ion of the aqueous humor.

3. Effect of Diamox upon the rate of transfer of bicarbonate ion into the posterior chamber.

4. Relation of the rate of transfer of bicarbonate ion into the posterior chamber with the rate of formation of aqueous humor.

5. Is the secretion of aqueous humor dependent upon the existence of an osmotic pressure gradient increasing from the plasma to the aqueous humor?

The results of the present investigation contribute significantly to the resolution of these five considerations.

1. *Source of bicarbonate ion.* In the previous paper[1] by us theoretical considerations and experimental evidence were adduced favoring a proposal that the major bulk of the bicarbonate ion in the posterior chamber fluid came from the bicarbonate ion in the plasma without the intermediary conversion to CO_2.

2. *Role of carbonic anhydrase in the elaboration of the bicarbonate ion.* A logical corollary of the results in the previous paper was that the formation of bicarbonate ions from the hydration of CO_2 contributed only a relatively small amount to the concentration in the aqueous humor of the posterior chamber. It therefore naturally follows from this that the carbonic anhydrase activity of the ciliary body apparently does not participate to any appreciable extent in the elaboration of the bicarbonate ion in the aqueous humor. Furthermore, as discussed in the next section of this paper, the rate of transfer of bicarbonate ion into the posterior chamber

was found to be markedly diminished within five minutes after the intravenous injection of Diamox, during which time the carbonic anhydrase activity of the anterior uvea was not appreciably affected.[7]

3. *Effect of Diamox upon the rate of transfer of bicarbonate ion into the posterior chamber.* It may be inferred from the foregoing discussions that although the bicarbonate ion may be transported into the posterior chamber predominantly in the form of the bicarbonate ion, rather than as a result of the carbonic anhydrase catalyzed hydration of CO_2 to carbonic acid, the possibility remains that the secretion of aqueous humor is essentially dependent upon the maintenance of an excess concentration of bicarbonate ion in the aqueous humor relative to that in the plasma, as first postulated by Kinsey.[10] Indeed, Becker[3] has attributed the decreased secretion of aqueous humor 30 to 60 minutes following the injection of Diamox to the accompanying decreased excess of bicarbonate ion concentration in the posterior chamber fluid relative to that in plasma. Green, Mann, and Kroman,[4] on the other hand, presented chemical data that were not completely compatible with such a correlation.*

In an attempt to clarify the Diamox picture it is worth while to reconsider some characteristics of the normal intraocular fluids. From the available information and based upon our present understanding, it is reasonable to state that the distribution of bicarbonate ion between the aqueous humor of the posterior chamber of the rabbit eye and the plasma is fundamentally a resultant of three component factors: (1) the rate with which the bicarbonate ion is transferred into the posterior chamber; (2) the loss or gain of bicarbonate within the posterior chamber; and (3) the rate with which the bicarbonate ion leaves the posterior chamber.† With regard to factor (2) the available evidence[11, 12] indicates that the lens probably does not contribute any appreciable amount of bicarbonate ion to the aqueous humor. On the other hand, the production of lactic acid from the metabolic activity of the lens may significantly neutralize part of the bicarbonate ion in the aqueous humor. The magnitude of this effect does not appear to be greater than 10 to 13 percent, and, indeed, probably lower.[11] It is also possible that the bicarbonate ion may diffuse into the vitreous humor, as has been suggested by Davson and Luck.[13] However, analysis of the vitreous humor of rabbit eyes by Green, Mann, and Sawyer,[14] following the continuous intravenous infusion of isotonic $NaHCO_3$, indicated that no apparent net exchange occurred between the bicarbonate ion in the aqueous humor of the posterior chamber and the vitreous humor (see also discussion in previous paper[1]). It is, therefore, reasonable to conclude that in the normal rabbit eye the concentration of bicarbonate ion in the posterior chamber fluid is maintained essentially by a balance between the rates of transfer into the eye and of flow out.

The question naturally arises as to the mechanism whereby Diamox causes a decrease in the PC/Pl ratio of concentrations of radioactive bicarbonate or in the total bicarbonate ion concentration, as manifested by its effect upon the above-described three

* Although the observations of Langham and Lee[6] on the dynamics of aqueous humor formation and intraocular pressure are not in accord with those of Becker,[3] they nevertheless are in agreement as to the significance of the maintenance of an excess bicarbonate ion concentration in the aqueous humor relative to that in the plasma. Their observations of the effect of Diamox upon the bicarbonate ion concentration of the aqueous humor of the anterior chamber in the first 15 minutes differ from those of Becker[3] and of Green and co-workers.[2]

† In view of the rapid transfer of the bicarbonate ion from the plasma into the posterior chamber against a concentration gradient, and the unidirectional bulk flow of fluid in the same direction, it is not very likely that an appreciable diffusion of bicarbonate ion out of the posterior chamber into the plasma takes place. Undoubtedly some leak may occur, but it is presumed that the major bulk of the bicarbonate ion leaves the posterior chamber by flow into the anterior chamber.

determinants. In the first place, the available evidence suggests that the intravenous injection of Diamox had no apparent effect upon the metabolic activity of the lens. Thus, Green and Bocher[15] have shown that the carbonic anhydrase activity of the lens remained unchanged and Green and Solomon[16] have demonstrated that with homogenates of lenses the presence of 1×10^{-3}M Diamox had no apparent effect either upon the hexokinase activity or upon the ability of the homogenate to produce lactic acid from glucose-6-phosphate under conditions of active lactic acid production. It is unlikely, therefore, that under the conditions of the experiments with Diamox the normal metabolic activity of the lens would be disturbed. It is possible, however, that the pH of the aqueous humor of the posterior chamber may be decreased as a result of the action of Diamox, thereby leading to a lowering of the bicarbonate ion in the aqueous humor of that chamber. Unfortunately, the effect of Diamox upon the pH of the posterior chamber fluid has not been measured so that any conclusions in this regard would appear to be unwarranted.* In any event it would appear logical to focus attention upon the effect of Diamox upon the rate of transport of the bicarbonate ion into the eye and the rate of outflow.

In this connection it is worth emphasizing that although the intravenous injection of a single dose of Diamox is known to cause a maximal decrease (21 percent) in the bicarbonate ion concentration of the posterior chamber fluid in about 60 minutes,[2,3] no direct experimental evidence has been adduced to show that the drug reduces the rate of transfer of the ion into the posterior chamber.† As noted earlier in this paper Diamox caused a 17 percent decrease in the concentration of radioactive bicarbonate ion of the posterior chamber fluid after 50 to 60 minutes, which was in good agreement with the nonradioactive chemical findings[2,3] that under similar experimental conditions the total bicarbonate ion concentration fell about 21 percent.

This correlation between the decrease in the concentration of total bicarbonate and of the radioactive bicarbonate supports the concept proposed in the previous publication[1] that the bicarbonate ion of the posterior chamber fluid arose chiefly directly from the blood bicarbonate. It may be argued, of course, that after 60 minutes of Diamox action, it may be assumed that steady-state conditions have been temporarily attained with regard to the bicarbonate ion concentration in the posterior chamber fluid and the decreased rate of formation of aqueous humor. Consequently, at this point, since the rates for the bicarbonate ion of transport into and of flow out of the posterior chamber are equal, the decreased concentration of the bicarbonate ion must have resulted from a decreased rate of transfer into the posterior chamber. Although this reasoning is not without validity, the experimental results with the longer term Diamox action, at best, is only indirect evidence suggesting that the action of the drug is to decrease the rate of transfer of bicarbonate ion into the eye.

If the mode of action of Diamox is indeed as suggested above, then it should be possible to measure a decreased rate of transfer of bicarbonate ion into the posterior chamber almost immediately or within a short time after the injection of the drug. The results of such short term experiments with C-14 labeled $NaHCO_3$ designed to test the effect of Diamox upon the turnover rate of bicarbonate ion in the aqueous humor are given in Table 5 and, for ease of comparison with the longer term effects of Diamox, are graphically illustrated in Figures 5 and 6.

* Green, Mann, and Kroman[3] found that Diamox had no appreciable effect upon the pH of the aqueous humor of the anterior chamber during the first few hours (maximum lowering of 0.05 pH units). Langham and Lee,[4] on the other hand, reported a drop of 0.1 to 0.2 pH units within 15 minutes.

† Langham and Lee[4] concluded from their studies of the dynamics and chemistry of the aqueous humor of the anterior chamber that there was no evidence that Diamox affected the rate of secretion of the bicarbonate ion into the rabbit eye.

The results clearly show that the action of Diamox was indeed very rapid, having caused within six minutes a marked lowering of the PC/Pl and AC/Pl ratios of concentrations of radioactive bicarbonate ion compared to values in normal untreated rabbits. It can also be seen that the effects of the short-term action of the drug on the PC/Pl ratio were even more pronounced than that caused by the drug injected 45 minutes prior to the injection of the labeled bicarbonate (a decrease of 31 percent compared with a decrease of 17 percent). In the case of the AC/Pl ratio while the effect of the short-term action of Diamox was only slightly greater than that of the longer term action, the results indicate an equally rapid effect of the drug upon the turnover rate of bicarbonate ion in the anterior chamber fluid.

The results of this investigation, therefore, clearly establish the fact that the intravenous injection of Diamox caused a marked decrease in the rate of transport of bicarbonate ion into the posterior chamber. The results further indicate that the drug also decreased the turnover rate of bicarbonate ion in the anterior chamber within the first six minutes, which strongly suggests that the bicarbonate ion from the plasma may be transported directly into the anterior chamber, rather than undergo merely passive diffusional exchange with that in the anterior chamber fluid, as postulated by Kinsey.[8]

Since the decrease of the transport of the bicarbonate ion from the plasma into the aqueous humor occurred within the first six minutes of the action of Diamox, during which time it had been shown that the carbonic anhydrase activity of the anterior uvea was inhibited only about 20 percent, it is apparent that the enzyme does not participate to any significant extent in the elaboration of the bicarbonate ion in the aqueous humor of the rabbit eye, or in the secretion of aqueous humor.

4. *Relation of the rate of transfer of bicarbonate ion into the posterior chamber with the rate of formation of aqueous humor.* Gloster and Perkins[17] have shown that the ocular hypotensive action of Diamox was elicited within a few minutes of its injection into rabbits. Although the effect of the drug upon the secretion of aqueous humor during the same time period was not determined, it is reasonable to assume that the flow of aqueous humor was also suppressed. Indeed, the probability that the immediate fall in intraocular pressure was determined completely by a decreased rate of formation of aqueous humor was concluded recently by Langham[18] who observed that the time course of the fall in the rate of aqueous humor formation immediately after the injection of Diamox agreed closely with the time course of the lowering of the intraocular pressure.

The results of this investigation, therefore, together with the observation of the above investigators, establish for the first time a definite correlation between the rate of transport of bicarbonate ion into the aqueous humor and the rate of formation of that fluid. Furthermore, it becomes apparent that while a decreased rate of secretion of aqueous humor is instantaneously translated into a similar decreased rate of flow out, a decreased rate of transfer of bicarbonate ion into the eye is not immediately accompanied by a disease in the bicarbonate ion concentration in the aqueous humor.

The reason for this is based upon the fact that the posterior chamber has a finite volume and, therefore, requires a finite time for the concentration of the bicarbonate ion therein to be lowered to that determined by the decreased amount entering per unit time. Indeed, the time required to reach this new equilibrium is fundamentally dependent upon the rate of flow of the aqueous humor. Since, under the circumstances, the latter was also decreased, a longer time is required for the decreased rate of transfer of bicarbonate ion into the posterior chamber to be reflected in a decreased bicarbonate ion concentration (about 60 minutes).

This is shown in Figure 5 where the initial PC/Pl ratio is a measure of the decreased

rate of transport of the labeled ion. The ratio gradually rose with time during the adjustment of the bicarbonate ion concentration to the reduced steady-state level and approached the value obtained after 60 minutes of Diamox reaction. At this point the PC/Pl ratio is a product of the decreased rate of transport of bicarbonate ions as well as the decreased rate of flow of aqueous humor. It is obvious, of course, that a decreased rate of secretion of aqueous humor itself, without an accompanying decrease in the rate of transfer of bicarbonate ion into the eye, would have no effect upon the bicarbonate ion concentration in the eye.

The results, therefore, clearly indicate that the decrease in the rate of transfer of the bicarbonate ion into the posterior chamber, and the decrease in the rate of formation of the aqueous humor, occurred prior to the decrease in the bicorbonate ion concentration. The latter event, therefore, was not the factor responsible for the decreased rate of formation of aqueous humor, but was itself the resultant essentially of a decreased rate of transfer of bicarbonate ion into the posterior chamber.

Accordingly, the results argue against the concept that the maintenance of an excess concentration of bicarbonate ion in the aqueous humor of the posterior chamber relative to that in the plasma is chiefly responsible for the secretion of aqueous humor. On the other hand, the results of this investigation cannot define precisely the functional significance of the active transport of bicarbonate ion and the resulting maintenance of an excess concentration in the aqueous humor relative to that in the plasma.

While it seems logical to suggest that the active transport of bicarbonate ion into the posterior chamber may be the primary event in the formation of aqueous humor, a definite conclusion in this regard must await accurate simultaneous determinations of the rate of formation of aqueous humor and rate of transfer of bicarbonate ion in normal rabbit eyes and at varying time periods after the administration of Diamox.

5. *Is the secretion of aqueous humor dependent upon the existence of an osmotic pressure gradient increasing from the plasma to the aqueous humor?* It is obvious from the above discussion that the rate of secretion of fluid into the posterior chamber of the rabbit eye is probably directly related to the rate of transfer of the bicarbonate ion, and, therefore, may not be dependent upon the existence of an osmotic pressure gradient from the plasma to the aqueous humor. Indeed, the recent osmotic pressure measurements of Kass and Green[19] indicate the absence of any significant osmotic pressure gradient between the aqueous humor of the posterior chamber and the plasma and show that the action of Diamox had no apparent effect upon this relationship. On the other hand, the measurements clearly established that the osmotic pressure of the aqueous humor of the anterior chamber was significantly greater than that of the plasma and of the aqueous humor of the posterior chamber. Furthermore, the intravenous injection of Diamox resulted in a significant decrease in the osmotic pressure gradient between the aqueous humor of the anterior chamber and the plasma as well as the posterior chamber fluid.

These osmotic pressure relationships provide a reasonable explanation for the apparent discrepancy, mentioned previously in this discussion, between the 30 percent decrease in the AC/Pl ratio of radioactivity concentrations and the 12 percent decrease in concentration of bicarbonate ion in the aqueous humor of the anterior chamber measured 60 minutes after the intravenous injection of Diamox. It will be recalled that in the previous paper it was suggested that the lower concentration of bicarbonate ion in the anterior chamber fluid compared to the concentration in the posterior chamber fluid could well be explained on the basis that the concentration of bicarbonate ion entering the

anterior chamber with the osmotic flow of fluid (solvent drag) from the plasma was lower than that flowing in from the posterior chamber.

Accordingly, since it has been shown by Kass and Green[19] that Diamox decreased the osmotic pressure gradient between the aqueous humor of the anterior chamber and the plasma, the resulting decreased flow of fluid into the anterior chamber would be accompanied by a decreased rate of entry of bicarbonate ion from the plasma. This could account for the observed decrease in the AC/Pl ratio of concentrations of radioactivity.

In addition, since the transport of fluid and bicarbonate ion from the plasma into the anterior chamber normally may be responsible for diluting the bicarbonate ion concentration entering from the posterior chamber,[1] a suppression of this transport, instead of causing a decrease in the concentration of bicarbonate ion in the anterior chamber fluid, would tend to make that concentration more directly a reflection of the concentration entering with the posterior chamber aqueous humor. Indeed, as demonstrated in an earlier publication,[2] during the first three hours of Diamox action this relationship obtained. Furthermore, since the volume of the anterior chamber is about five times that of the posterior chamber,[20] a longer time is required for a concentration change in the posterior chamber to be manifested in the anterior chamber.[2]

These considerations, therefore, provide a reasonable explanation for the measured 30 percent decrease in the AC/Pl ratio of concentrations of radioactive bicarbonate ion and only a 12 percent decrease in the concentration of bicarbonate ion in the anterior chamber aqueous humor 60 minutes after the injection of Diamox.

The results from this laboratory, therefore, indicate the existence of an osmotic flow of fluid from the plasma into the anterior chamber and suggest that the flow of aqueous humor out at the angle of the rabbit eye is composed of at least two components: (1) the flow into the posterior chamber which then passes through the pupillary space into the anterior chamber; and (2) an osmotic flow from the plasma into the anterior chamber. The absence of a significant osmotic pressure gradient between the plasma and the aqueous humor of the posterior chamber suggests that the flow of fluid into the posterior chamber may not be an osmotic flow. Whether this transport of water is functionally related to the active transport of the bicarbonate ion or some other ion, or is dependent upon some other mechanism, remains to be determined. The measurement of the relative contributions of the abovementioned two sources of aqueous humor flow to the dynamics and chemistry of the aqueous humor in the anterior chamber under normal conditions and after the administration of Diamox represents a stimulating and interesting new approach to aqueous humor studies.

It would seem that a logical approach to future investigation of the kinetics of aqueous humor formation and flow should include the simultaneous use of deuterium or tritium labeled H_2O and C-14 labeled $NaHCO_3$ as tracers of the dynamic properties of the fluids in both the anterior and posterior chambers relative to that in the plasma.

Summary

The effects of an intravenous injection of Diamox (30 mg./kg.) upon the kinetic properties of the bicarbonate ion in the aqueous humors of the rabbit eye were studied with the use of intravenously administered C-14 labeled sodium bicarbonate ($NaHC^{14}O_3$).

Two time periods of action of Diamox were studied: longer duration, 50 to 60 minutes; shorter duration, five to 10 minutes.

After 50 to 60 minutes of Diamox action the ratio of the concentration of radioactive bicarbonate in the aqueous humor of the posterior chamber to that in the plasma

(PC/Pl) was decreased by 17 percent. In view of the absence of any significant effect of Diamox upon the kinetics of the C-14 labeled bicarbonate ion in the plasma, this lowering of 17 percent reflected the decrease in the *concentration* of radioactive bicarbonate ion in the posterior chamber fluid and was in good agreement with the previously found 21 percent decrease in the total bicarbonate ion concentration. This good correlation supports the concept, previously proposed by us, that the bulk of the bicarbonate ion in the posterior chamber fluid is derived from the bicarbonate ion in the plasma.

The decrease in the ratio of concentration of radioactive bicarbonate ion in the aqueous humor of the anterior chamber to that in the plasma (AC/Pl), 50 to 60 minutes after the injection of Diamox, was about 30 percent. This suggests that Diamox suppressed the rate of transfer of the bicarbonate ion into the anterior chamber directly from the plasma.

The shorter duration experiments (five to 10 minutes of Diamox action) were designed to determine the effect of the drug upon the rate of transfer of the bicarbonate ion into the aqueous humor before a new steady-state distribution of the bicarbonate ion concentration had been established, as was the case in the longer duration experiments.

The rate of transfer of the bicarbonate ion from the plasma into the posterior chamber was lowered by 30 percent five minutes after the injection of Diamox. Since it had been shown by other investigators (Gloster and Perkins and Langham) that the rate of flow of aqueous humor was decreased within a few minutes of the injection of Diamox, the results of the present investigation established for the first time a definite correlation between the rate of transport of the bicarbonate ion and the rate of flow of aqueous humor.

In view of previous findings by Green, et al., that the carbonic anhydrase activity of the anterior uvea had been inhibited less than 20 percent five minutes after the injection of Diamox, it is apparent that the enzyme does not participate to any significant extent in the elaboration of the bicarbonate ion in the posterior chamber or in the rate of flow of aqueous humor.

In view of the longer time (about 60 minutes after the injection of Diamox) required for the maximum decrease in total bicarbonate ion concentration of the posterior chamber fluid to occur, it is reasonable to conclude that the rate of flow of aqueous humor is not fundamentally dependent upon the maintenance of the normal excess of bicarbonate ion concentration in the posterior chamber relative to that in the plasma. Rather it is reasonable to suggest that the rate of formation of the aqueous humor in the posterior chamber may be directly related to the rate of transfer of the bicarbonate ion into that chamber.

After five to 10 minutes of Diamox action the ratio of the concentration of radioactive bicarbonate of the aqueous humor of the anterior chamber to that of the plasma was lowered by some 30 percent. The reason for this marked effect was discussed on the basis of the findings of Kass and Green that the aqueous humor of the anterior chamber was significantly hypertonic to the plasma and to the aqueous humor of the posterior chamber.

The results, therefore, indicate that the flow of aqueous humor out of the anterior chamber at the angle is composed essentially of an osmotic flow from the plasma into the anterior chamber and the unidirectional flow from the plasma into the posterior chamber, the latter being related to the rate of transfer of the bicarbonate ion.

The intravenous injection of Diamox has been shown to cause a significant lowering of the rate of transfer of the bicarbonate ion from the plasma directly into the anterior and posterior chambers of the rabbit eye.

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SCOTOPIC AND PHOTOPIC THRESHOLDS OF THE PUPILLARY LIGHT REFLEX IN NORMAL MAN*

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The pupillary light reflex and visual perception share a large portion of their afferent path, from the retina via the optic nerve and chiasm to the posterior third of the optic tract. Similarities or differences between the pupillomotor effect and the subjective sensation evoked by light stimuli of various intensities, colors, areas, durations, and frequencies might therefore shed some light upon some of the still controversial questions concerning the basic sensory mechanisms. Clinically, such studies may lead to the objective verification of suspected damage in the retina, optic nerve, chiasm, or tract.

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Considerable work has been done during the past 60 years with the aim of relating pupillary and visual events. The results have been widely divergent, which is not surprising when we consider the differences in experimental conditions from author to author, and the difficulty inherent in observing an organ as small and as mobile as the iris.

In the older work, some form of illumination had to be provided to allow the iris to be seen. Flash photography and, more recently, infrared photography and infrared cinematography, infrared-sensitive photoelectric devices, and the infrared-sensitive image-converter tubes have been used to observe the pupil in darkness. Some authors examined the pupillary size after adaptation to various lights while others drew their conclusions from the rate of pupillary redilatation after the light stimulus was withdrawn. Still others studied the transient pupillary responses to intermittent light stimulation of the dark-adapted eye, the reactions of the pupil to an increment or decrement of a given level of constant illumination, or the effect of changes in color or in retinal position of the light stimulus.*

Since the completion of our electronic pupillography,[1] we have been able to obtain accurate, continuous traces of the pupillary diameter and reactions to light or other stimuli in total darkness or at any level of light adaptation, without the time-consuming measuring of thousands of pupil images per experiment which was needed in the earlier cinematographic technique. We therefore embarked upon a study of the relations between pupillary responses and visual sensation, which had heretofore appeared too extensive a task to undertake.

As a first step in this direction, we examined the pupillary responses of the dark-adapted eye to intermittent light stimuli of threshold intensity.

* A comprehensive review of these various methods is in preparation. For this reason, a detailed discussion of the literature is omitted in the present paper.

Methods

In all our experiments, the light scanning beam needed for pupillographic recording was passed through a Wratten infrared no. 87 filter. Since our scanning beam is very small, and since it is present at any one point of the eye only a minute fraction of the time, this light could not be seen, even after full dark adaptation.

For stimulation, we used the Sylvania glow modulator tube which furnishes white light. With a condensing lens of 31 mm. diameter and 31 mm. focal length, placed 35 mm. from the mica window of the tube, a slightly divergent beam of about 20 mm. diameter at the level of the subject's cornea was obtained. We used three different stimulus durations, namely 7.5 milliseconds, 0.1 second, and 1.0 second. The shortest stimuli were electronic square-wave flashes, using 100 ma. across the tube, while the longer ones were composed of trains of identical flashes at 100 cycles per second. Such fast trains of flashes appear as a steady light to

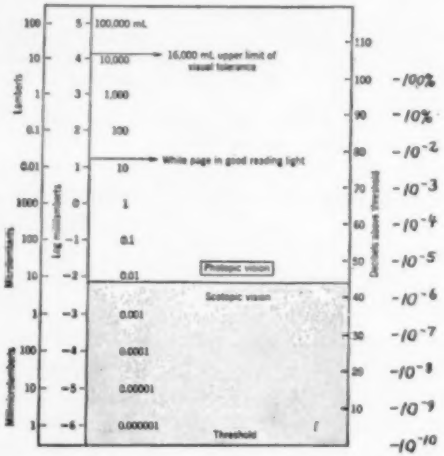

Fig. 1 (Lowenstein and Loewenfeld). *Approximate brightness of light stimuli.* Figure 14 of Bartley (1951): The psychophysiology of vision. (In Stevens, S. S.: Handbook of Experimental Psychology. New York, Wiley, 1951, p. 945.) On the right side, the scale of approximately comparative brightness levels, used in our experiments, is added. Further explanation in the text.

the subject, while the tube can be run at a higher brightness than would be possible with a D.C. source.

To avoid a change in the color and time characteristics of the light pulses, the intensity of the stimulating light was varied with Wrattan neutral gray filters, graded in logarithmic steps from full intensity down to $10^{-11\text{th}}$ power. With the conditions described above, the normal visual threshold for one-second stimuli ranged from about $10^{-9.3}$ to slightly lower than 10^{-10} on this arbitrary scale (fig. 1).*

After 25 to 30 minutes of dark adaptation, the visual threshold of each subject was determined within one log unit of stimulus intensity. For example, when the stimulating light was not seen with the 10^{-11} filter but 10 out of 10 flashes with the 10^{-10} filter were seen, we considered the threshold to lie roughly between these values and called it 10^{-10+}. We then recorded the pupillary activity while series of 10 such threshold light stimuli were presented, with the 7.5-millisecond stimulus-series recorded first, then the 0.1-second series, and finally the 1.0-second series. The same procedure was repeated at the next highest log unit of light intensity, and so forth, up to full intensity. Between stimulus series, periods of readaptation were interposed, namely two minutes at the low levels of stimulation (up to 10^{-6}) and four minutes at the brighter levels.

The complete series of experiments was done on five normal subjects, 16 to 38 years of age. In an additional nine subjects of various ages, parts of the series were recorded. Further series of experiments were done on seven subjects, whereby blue and red monochromatic filters were added to the stimulating light.

* The stability of this light is excellent. Because of its properties as a flashing light we are, however, at present unable to obtain an accurate measurement of its intensity. Until such measurements can be obtained, we will indicate light intensity in terms of (1) the arbitrary scale of gray filters used in our experiments, and (2) logarithmic units above absolute visual threshold of each subject.

Results

From subject to subject, the visual threshold varied slightly. While two of our chief subjects could just see 10 out of 10 flashes at 10^{-10}, the remaining three could not. At the visual threshold level of each subject, the pupils did not respond, but they contracted in all cases at the next highest log unit of intensity. The amplitude of the threshold responses varied from subject to subject, in the same subject from time to time, and within the same series from reaction to reaction, but the light increment above visual threshold which was needed to bring the pupils into play remained within one log unit for all normal subjects.

The first line of Figure 2 shows four normal pupillary contractions, elicited by four 1.0-second light stimuli two log units brighter (10^{-8}) than the visual threshold of the subject (10^{-10+}). But the pupil does not always respond as regularly. Threshold reactions to light are delicate and are easily suppressed by interfering sensory or psychologic stimuli. Under the influence of such interfering stimuli, cortico-thalamic-hypothalamic mechanisms are brought into play; they affect the pupil, firstly, by inhibiting the oculomotor nucleus and secondly, by sending active pupillary dilator impulses to the iris via the cervical cord and peripheral sympathetic chain.[2]

Both these influences are detrimental to the development of a contraction to light. For example, in Figure 3, the upper line shows three threshold contractions to light which were elicited while the subject, who happened to be very tired, was gradually falling asleep. During this phase of decreasing supranuclear inhibition the contraction to light was enhanced and redilatation absent. At the end of the third stimulus, the subject closed his eyes and was awakened by a psycho-sensory stimulus shortly before the fourth light stimulus. During the phase of awakening the pupils dilated vigorously. The light reflex was suppressed but reappeared in

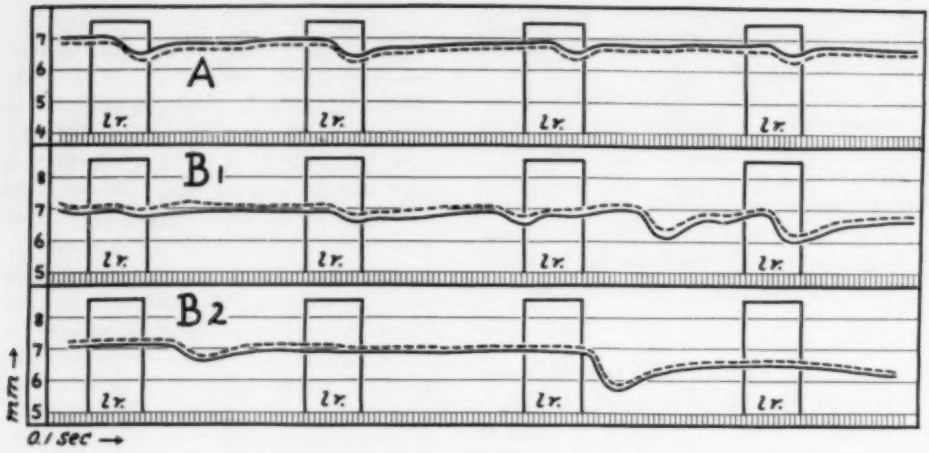

Fig. 2 (Lowenstein and Loewenfeld). *Reactions to light stimuli of threshold intensity, and spontaneous pupillary contractions.* Pupillary diameter is plotted as the ordinate (in mm.) against time as the abscissa (in 0.1 sec. units, indicated by the small vertical divisions at the bottom of each line). The solid line represents the right, the broken line the left pupil. The subjects' eyes were in total darkness, except for the times outlined by the frames *l.r.*, when the right eye was exposed to light stimuli of one second duration and of low intensity.

First line (A): Four reactions to light. The stimulus intensity (10^{-5}) was two log units brighter than the subject's visual threshold ($10^{-3.0}$). After a latency period of about 0.45 second, the pupils contracted an average of 0.6 mm., whereby the direct and consensual responses were equal. The duration of the shallow contractions was short, so that pupillary redilatation began before the light stimuli had come to an end.

Second line (B1): Pupillary contractions shown by a second subject. The four light stimuli (10^{-5}) were about one log unit brighter than the subject's visual threshold ($10^{-3.6}$). Only the first, second, and fifth contractions could be caused by light stimuli. The third contraction had reached its peak at the beginning of the third stimulus, and the fourth contraction occurred in darkness, between stimuli.

Third line (B2): Pupillary behavior of subject B during light stimulation of visual threshold intensity (10^{-5}). Two pupillary contractions occurred, more than a full second after the first and the third light stimuli. They were spontaneous movements, associated with involuntary, small blinks. The light stimuli remained ineffective.

its normal shape a few seconds later, in response to the fifth light stimulus.

On the other hand, many subjects show spontaneous pupillary contractions, even in total darkness. Most of these movements are associated with involuntary eye movements or blinks. Without continuous, accurate recording and an event marker, many of these spontaneous oscillations are indistinguishable from the threshold contractions to light. For example, the second line of Figure 2 again shows four light stimuli, but there are five pupillary contractions. Upon closer examination it is evident that the third and fourth contractions were not caused by a light stimulus. The third contraction had already reached its peak at the beginning of the third light stimulus and the fourth contraction occurred between light stimuli.

In the third line of Figure 2 four stimuli and two pupillary contractions can be seen. Some observers would consider such a situation a pupillary threshold. Both these contractions began, however, more than a full second after the beginning of the light stimulation and could not have been caused by the light at all.

The most striking features of the pupillary threshold reactions, when compared to pupillary reactions elicited by brighter light, were a long latency period, small extent, and short duration. While the normal reflex to bright

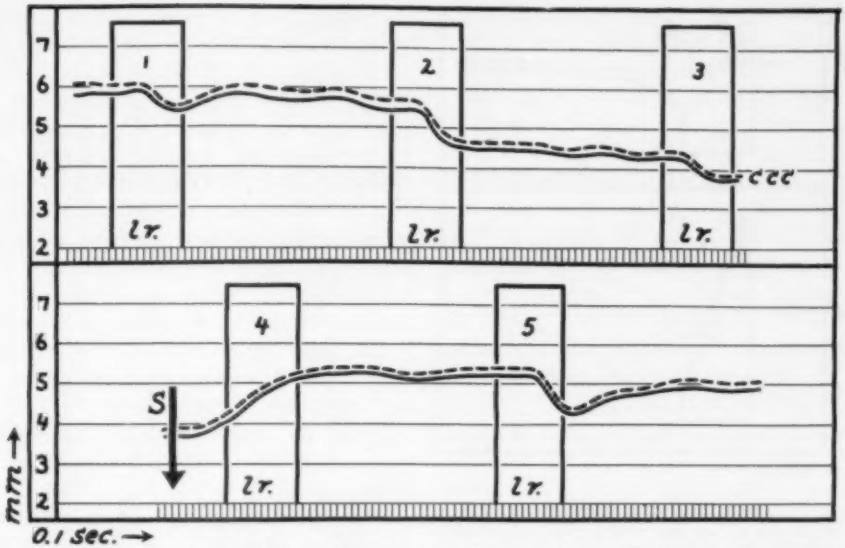

Fig. 3 (Lowenstein and Loewenfeld). *Enhancement and suppression of low intensity light reflexes by central inhibitory mechanisms.* Pupillary diameter is plotted as the ordinate (in mm.) against time as the abscissa (in 0.1-sec. units), whereby the solid line represents the right, the broken line the left pupil. The stimulus light intensity (10^{-1}) was about two log units brighter than the subject's visual threshold (10^{-3*}).

First line: Three reactions to light, elicited while the subject gradually fell asleep. During this phase of decreasing supranuclear inhibition, the pupillary contraction was enhanced, redilatation absent. At the end of the third light stimulus, the subject closed his eyes (*c c c*).

Second line: Shortly before the fourth light stimulus, the subject was awakened by a verbal stimulus ("wake up!"). During awakening, the pupils dilated and the fourth light reflex was suppressed. The fifth light stimulus elicited the normal threshold response of the awake subject.

light begins about 0.2 to 0.3 second after the beginning of stimulation, the latency period of the threshold contractions ranged between 0.45 and 0.6 second, that is, about twice as long.* The contractions were shallow and usually did not last a full second. Even in the continued presence of light stimulation, the pupils began to redilate. In other words, prolonging the stimulus beyond 0.1 second did not prolong the pupillary contraction.

In marked contrast to this inability to prolong the response at low light intensity, a bright, long stimulus results in a far longer and more extensive contraction than a bright, short stimulus. In Figure 4, (A) shows the short-stimulus and long-stimulus responses at low intensity, B those at high intensity. The short-stimulus reactions are presented by the broken lines, the long-stimulus responses by the solid lines. The long and short low-intensity reactions were identical. Both showed the typical long latency period, slow, inextensive contraction, and short duration of such threshold reactions: In contrast, the high-intensity reactions showed a shorter latency period, faster, more extensive contraction, and a very great increase in duration and amplitude of the reaction upon longer stimulation.

The pupillary effects of an increase of stimulus duration and an increase of stimulus intensity are therefore different in the low-intensity and the high-intensity range. In the

* The threshold pupillary contractions are small in extent and begin very gradually. The true latency period may be slightly shorter than indicated, because in our recording system, a pupillary movement of less than ±0.025 mm. cannot be recorded. We therefore do not claim absolute accuracy in this respect.

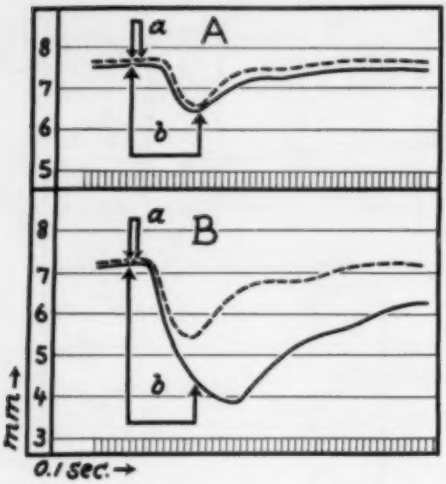

Fig. 4 (Lowenstein and Loewenfeld). *Reflex characteristics at low and at high levels of stimulus intensity.* Pupillary diameter is plotted as the ordinate (in mm.) against time as the abscissa (in 0.1-sec. units). The broken lines indicate the pupillary responses to short light stimuli (1/10 second, outlined by double-arrows *a*), the solid lines reactions to longer light stimuli (one sec., outlined by double-arrows *b*).

(A) *(first line):* Low intensity reactions, elicited by light stimuli three log units brighter (10^{-1}) than the subject's visual threshold ($10^{-4.0}$). The short-stimulus and long-stimulus reactions were alike. Both showed the long latency period, low peak speed, small extent, and short duration typical for low-intensity reactions.

(B) *(second line):* Pupillary reactions of the same subject to bright light (10^{-1}). Short- and long-stimulus reactions both showed a shorter latency period, faster peak speed, and greater extent than the low-intensity responses of line A. The long, bright stimulus caused a continued, extensive contraction far greater than the one elicited by the short, bright stimulus.

low-intensity range, an increase in light intensity causes a greater pupillary contraction than an increase in stimulus duration. Conversely, in the high-intensity range a longer, dimmer stimulus brings about a much longer, more extensive pupillary contraction than a shorter, brighter stimulus.

All these differences in the reflex characteristics between the low-intensity and the high-intensity pupillary responses demonstrate again that it is idle to make blanket statements on the so-called "normal pupillary reflex to light." Like all other reflexes, this response varies in amplitude, speed, latency period, and duration, according to the conditions of the experiment. Therefore, if we wish to define the normal reflex shape and to distinguish it from the pathologic, we have to adhere to rigidly controlled sets of experimental conditions, and we have to realize that any change in these conditions will cause a corresponding change in the reflex pattern.

Since the threshold pupillary contractions in our experiments occurred at only a single logarithmic step above the visual threshold, we concluded that they must be rod responses. To further prove this assumption, we studied the pupillary threshold during dark adaptation.

We exposed the subjects' eyes for two minutes to bright light (25 percent of total intensity) and then elicited series of low-intensity reactions after one, six, and 30 minutes of dark adaptation. In Figure 5, the extent of pupillary contractions, evoked by light stimuli of two brightness levels, are shown. The black columns represent responses to a stimulus intensity of threshold value (10^{-9}, one log unit above the visual threshold of the subject), the gray columns those to stimuli 100 times brighter (10^{-7}). For each series of reactions, the 10 individual contractions and the average of all 10 reactions are shown.

After only one minute of dark adaptation, the pupil failed to respond to the dimmer stimuli but contracted to an average of about 0.2 mm. in response to the brighter stimuli (fig. 5, first line). As is usual for threshold reactions, the amplitude of the contractions varied irregularly and the first response was often not the largest one.

After six minutes of dark adaptation, the dim stimuli had become effective (fig. 5, second line). The reactions averaged a little less than the reactions to the brighter stimuli had done before. The six minutes of dark adaptation had therefore lowered the pupillary threshold by a little less than two log units.

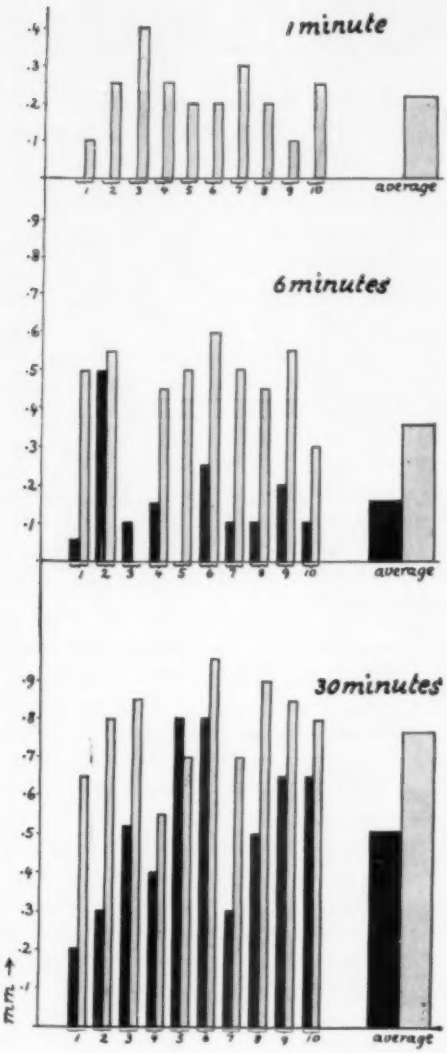

Fig. 5 (Lowenstein and Loewenfeld). *Effect of dark adaptation upon the extent of low intensity light reactions.* In this experiment, series of 10 low-intensity light stimuli were presented one, six, and 30 minutes after preadaptation to bright light (25 percent of full intensity, two minutes). The black columns indicate the extent of pupillary contractions to light stimuli of threshold intensity (10^{-8}, that is, one log-unit above the subject's visual threshold [10^{-9}]). The gray columns show the extent of contractions to light stimuli 100 times brighter (10^{-3}). In each line, the extent of the individual responses of each series is shown at the left (numbered 1 to 10), the average of each series of 10 reactions on the right.

First line: After only one minute of darkness, the 10^{-8} stimuli remained ineffective. The pupils contracted an average of 0.2 mm. in response to the brighter stimuli (10^{-3}).

Second line: After six minutes of dark adaptation, the pupils reacted to the dim stimuli. The responses were somewhat less extensive than the brighter-stimulus reactions had been after one minute of darkness.

Third line: Prolonged dark adaptation brought about an additional improvement of the reactions. The dim-stimulus series became more extensive than the brighter-stimulus series had been after only six minutes of dark adaptation.

An additional, great improvement was brought about by further dark adaptation. The dim-stimulus reactions became more extensive than the brighter-stimulus reactions had been after only six minutes of dark adaptation (fig. 5, third line). The pupillary threshold had therefore been lowered again, equivalent to somewhat more than two log units of stimulus intensity. This additional threshold-lowering effect of prolonged dark adaptation characterized the pupillary threshold contractions as rod responses.

But what about the cones? They have their own pupillary threshold. Similar to the pupillary rod-threshold, the pupillary cone-threshold was found to be silghtly above the threshold for cone vision. Figure 6 demonstrates the extent of the pupillary contractions of a normal subject which were elicited by light stimuli of increasing brightness, from the absolute threshold to full intensity. The subject's absolute visual threshold was slightly lower than 10^{-10}, the threshold of photopic vision approximately at 10^{-6}. Below this level of intensity, red was not seen and blue appeared gray.

Pupillary threshold reactions appeared at 10^{-9} and increased gradually over the next two logarithmic brightness steps. There was a sudden break in the line of increasing contraction extent, just above the threshold of photopic vision. Above this point, an increase of stimulus brightness in log-unit steps resulted in much steeper, fairly linear steps of increasing contraction extent. The line flat-

tened out when the motor capacity of the iris was approached (at about 3.75 mm. contraction in this subject).

When the duration of the responses was plotted instead of the extent (fig. 7), the break at the photopic brightness threshold was even more pronounced. Below this level of intensity, all stimuli had about the same duration. In contrast, the latency period declined steadily from threshold to maximal stimulus intensity.

While all our subjects showed this sudden increase in the pupillary effect of light bright enough for color vision, the relative extents of the low-intensity and high-intensity reactions varied greatly among individuals. In these variations, we found the deciding factor to be the degree of supranuclear inhibition present at the moment of stimulation (see above).

In earlier work[3] we demonstrated that subjects with increased supranuclear inhibition show less extensive reactions to weak light stimuli than subjects with relatively weak central inhibitory activity. Conversely, factors which reduce the degree of central inhibition, such as fatigue of the individual or the presence of a constant, low level of background illumination, tend to improve the low-intensity light reflexes in subjects with strong central inhibition while they depress the responses in subjects with weak central inhibition.

Figure 8 serves to demonstrate these facts. The curves with dots and solid lines were obtained from a normal young woman of 24 years of age (same subject as in figs. 6 and 7). Figure 8A shows, again, the increasing extent of contractions evoked by light stimuli of increasing brightness. In Figure 8-B, the solid line shows the same subject's first, second, and ninth pupillary reflexes, elicited by a series of bright light stimuli (25 percent of full intensity and one second duration). Previous work has established this reflex form as normal, under the conditions of the experiment.*

* For recent summaries of pupillographic results pertaining to this and the following statements, see[1, 4].

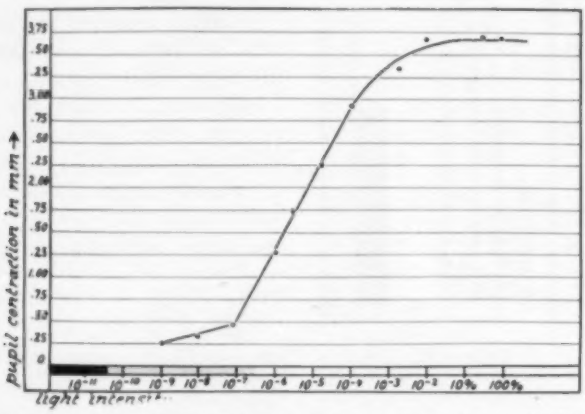

Fig. 6 (Lowenstein and Loewenfeld). *Extent of pupillary contractions, elicited by light stimuli of one-second duration and of increasing intensity.* The extent of pupillary contractions is plotted as the ordinate (in mm.) against stimulus intensity as the abscissa (in log units of brightness, with 100 percent the full intensity of the light source). The black area on the abscissa indicates values below the absolute visual threshold of the subject, the gray area the range of scotopic vision.

Pupillary reactions first appeared at the brightness level of 10^{-9}. With increasing stimulus intensity, the pupillary contractions became larger. Within the scotopic brightness range (up to about 10^{-6}), this increase in extensiveness was very gradual, but at the photopic level, a sudden break was observed in the line. The extent of pupillary contractions grew rapidly, in fairly linear steps, until the line flattened out when the motor capacity of the iris was approached (at about 3.75 mm. of contraction in this subject).

In contrast to the rather placid temperament of this subject, our second normal subject (crosses and broken lines) was a tense, excitable young woman 22 years of age. She was often emotionally agitated and felt irritable and restless for days, without apparent cause. When excited, she had an increased heart rate, her hands trembled, and she felt shaky. The pupillogram of Figure 8-B shows the relatively large initial diameter of the pupil in darkness and the slightly inhibited contractions to light, compared to the first subject, which are usually found in such subjects. Figure 8-A demonstrates that this subject's contractions to low intensity light stimuli were less extensive than those of the first subject, and more light was needed to reach full contraction.

The third subject of Figure 8 (dotted lines and squares) was a patient with a diencephalic lesion which interrupted the inhibitory path from the interbrain to the oculomotor nucleus. Clinically, the patient was suffering from essential hypertension and paroxysmal tachycardia. The pupillogram (fig. 8-B) shows the small initial pupillary diameter in darkness, the fast, squareshaped "tonohaptic" light reflexes, and the uniformity of the reflexes, which are typical for cases with damage to the supranuclear inhibitory system.

The visual and pupillary thresholds of this patient were about one log unit higher than those of the normal subjects. This fact may be explained in part by the smaller pupil which admitted only about one half as much light as the normal pupil, and partly by the patient's age (71 years) and condition.

The patient's threshold light reflexes were as extensive as those of the normal subjects, but the maximal contractions were less extensive, mainly owing to the small initial diameter of the pupil. In addition, only 1/1,000 of the available light intensity was needed to reach the maximal pupillary effect. The span between pupillary threshold and maximal pupillary effect was therefore only five log units when the supranuclear inhibition was missing, while it was seven log units in the well-balanced normal and eight log units in the hyperexcitable normal subject.

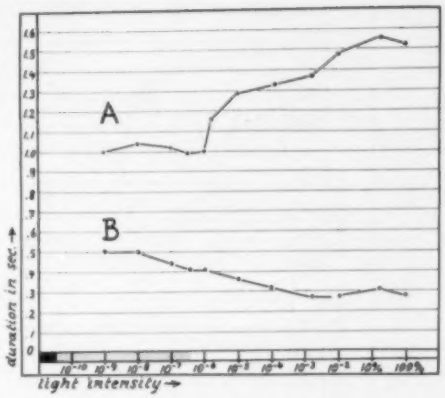

Fig. 7 (Lowenstein and Loewenfeld). *Latency period and duration of contractions to light, elicited by stimuli of one second-duration and increasing intensity.* Latency period (B) and duration of contraction (A) were plotted as the ordinate (in seconds) against stimulus intensity as the abscissa (in log units of intensity, with 100 percent the full brightness of the light source). The black area on the abscissa indicates light below the absolute visual threshold of the subject, the gray area the range of scoptopic vision.

(A) The duration of contraction was measured from the beginning of the stimulus to the beginning of pupillary redilatation. In the scotopic intensity range, all reactions were short and of uniform duration. When the stimulus intensity exceeded 10^{-4}, the contractions lasted longer. This increase in duration appeared suddenly, with further prolongation until a maximal duration for the subject was approached.

(B) In contrast to the sudden break in the lines of increasing duration and extent of the contractions (cf. A and fig. 6), the latency period was found to decline gradually from threshold light intensity to a minimal value (about 0.27 sec. in this subject).

To test our assumption that the sudden increase in duration and amplitude of the pupillary reactions to light stimuli above the photopic brightness level was due to the addition of the cone system and not to some quantitative factor more central in the reflex arc, we added colored filters to our stimulating light.

In Figure 9, the dots and solid line again represent the reactions to white light. When a monochromatic blue filter was interposed, the visual and pupillary sensitivity dropped

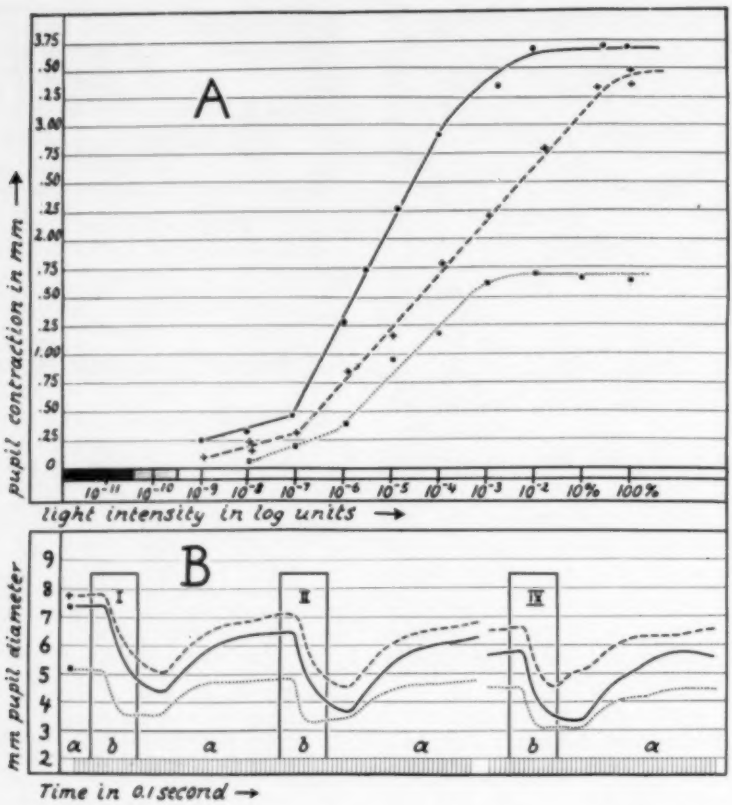

Fig. 8 (Lowenstein and Loewenfeld). *Reactions to light stimuli of increasing intensity in three subjects with different degrees of supranuclear inhibition. First line (A):* The extent of pupillary contractions is plotted as the ordinate (in mm.) against stimulus intensity as the abscissa (in log units of brightness). The dots and solid line represent the values of a normal subject (same as used for figs. 6 and 7), the crosses and broken line those of a second normal subject with functionally increased supranuclear inhibition, and the squares and dotted line those of a patient with damage to the supranuclear inhibitory system. The black area on the abscissa indicates values below the visual threshold of the normal subjects, the shaded area values below the visual threshold of the patient.

Second line (B): The first, second, and ninth of series of light reflexes of the same three subjects, elicited by bright light (25 percent of full intensity, one second). The curves demonstrate the differences in initial pupillary diameter in darkness, and the speed, extent, shape, and uniformity of the reactions (further explanation in the text).

about one logarithmic step, because the filter absorbed considerable energy. But otherwise, the blue-light and white-light functions were quite alike (crosses and broken line). This was not the case when a red filter was added (squares and dotted line): The pupillary theshold was raised to a little less than one log unit above that of photopic vision. In contrast to the blue-light and white-light functions, the red-light function rose sharply, in large steps, and did not show the initially small increase or the sudden break in the line of increasing contraction extent.

We conclude that both rods and cones furnish afferent impulses for the pupillary reflex to light. Just as for vision, the cone function and the rod function have separate thresholds. The cones, though less sensitive,

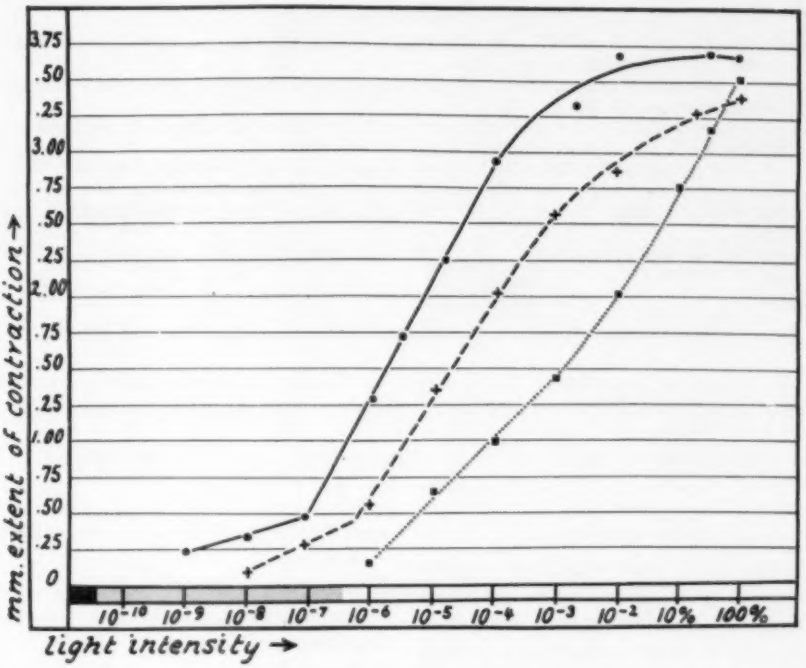

Fig. 9 (Lowenstein and Loewenfeld). *Extent of pupillary contractions, elicited by white, blue, and red light stimuli of one-second duration and of increasing intensity.* The extent of pupillary contractions is plotted as the ordinate (in mm.) against stimulus intensity as the abscissa (in log units of brightness). The black area on the abscissa indicates values below visual threshold of the subject, the gray area the range of scotopic vision. To match the brightness of the blue and red monochromatic filters used in this experiment (by the flicker fusion method), an additional 32.5 percent neutral gray filter was added to the red filter.

Solid line and dots: Curve for white light (cf. fig. 6).

Broken line and crosses: Curve for blue light. Due to the absorption of energy by the blue filter, the curve was shifted one log unit to the right. Otherwise, the white-light and blue-light curves were very similar.

Dotted line and squares: Curve for red light. Pupillary contractions did not appear until a brightness level slightly above the photopic visual threshold was reached. In contrast to the blue-light and white-light curves, the extent of contraction to red light increased sharply, in large steps. The gradual increase for the first three log units of intensity, and the sudden break in the line were missing.

are more effective than the rods in producing a fast and prolonged pupillary response.

The present findings are only a small step into the vast field of possible investigations of this kind. Because of the small number of subjects used in this study, and the lack of refinement of our stimulus conditions as to intensity and retinal area, no quantitative accuracy is claimed in these respects. The results prove, however, that the pupillary apparatus is sufficiently sensitive to make it a useful indicator of retinal function. In addition, the pupillary reactions have the advantage of being quantitatively objective. We would like to stress, however, that in view of the delicacy of the responses, and the frequent occurrence of spontaneous pupillary oscillations, accurate, continuous registration and an event marker are, in our opinion, absolutely essential. Without them, we are in danger of substituting the experimenter's subjective threshold of observation for the objective event.

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EXPERIMENTAL PRODUCTION OF OCULAR TUMORS*

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Introduction

Seligman, et al. (1939[1]), produced the first experimental brain tumors by the intracerebral implantation of methylcholanthrene pellets in mice. Various modifications of their technique were adapted to the mouse eye in an attempt to produce retinal tumors which might then be subjected to hyperoxia experiments. Tumors of most ocular structures, with the notable exception of the retina, were produced in this pilot study. One interesting observation, the downgrowth of surface epithelium through a small limbal puncture wound to line the anterior chamber following methylcholanthrene implantation, has already been reported.[2] The various ocular tumors that were produced are presented.

Subjects and Methods

Eyes of young mice of C_{57}, CF_1, A, A/Jax, C_3H (NIH), and C_3H (Cumberland Farms) were treated with 20-methylcholanthrene in various ways.

1. Injection in situ (A. A/Jax, CF_1)

Specially prepared no. 24 needles of short bevel and with fitted stylet were packed with methylcholanthrene (hereafter MCA) by pressing the tip of the needle forcibly into a pool of MCA crystals in a paraffin block (fig. 1). The needle was wiped free of fluorescing material and inserted directly into the anterior chamber through the cornea or into the posterior segment via a puncture one mm. posterior to the limbus; the "pellet" was ejected by pressing and locking the stylet, and the needle withdrawn (fig. 2). Injections were done in ultraviolet light (Woods lamp) and placement of the pellet in either locale was readily visualized. No effort was made to insure quantitative uniformity of the "pellet," but only injections in which the fluorescence was observed to cover at least a quarter of the segment injected were considered successful.

Some of the animals were first denuded of surface epithelium to an area ×3 the size of the puncture wound by scraping or (in the region of the conjunctiva) cutting away the epithelium and washing the area with two swabbings of 70 percent alcohol. Injec-

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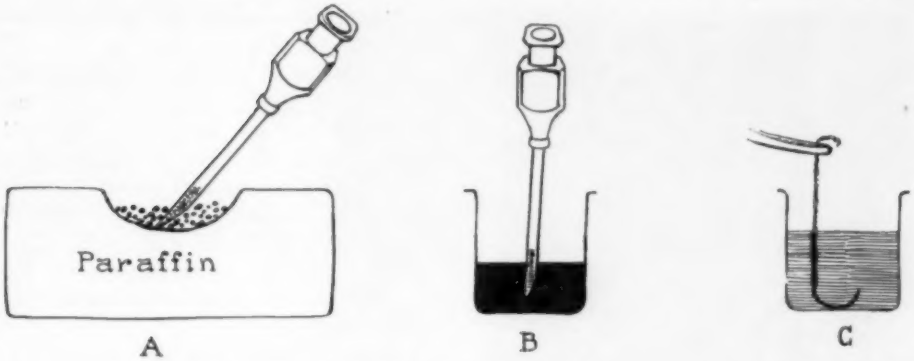

Fig. 1 (Patz, Wulff, and Rogers). Methods of preparing methylcholanthrene for introduction into the eye. (a) The dry crystals are packed into the end of hypodermic needle. (b) The molten methylcholanthrene fills the lumen of the hypodermic needle. (c) Atraumatic needle and silk suture are immersed in molten solution of MCA and cholesterol.

tion then followed in the manner outlined above. Controls were "injected" with a clean needle of identical size.

The right cornea of each of 12 animals was scraped of epithelium and MCA applied to the abraded area. The lids were sutured shut.

2. Irradiation and injection (A/Jax)

Another series of animals five to eight weeks old were operated with the injection technique coupled with two exposures to X rays, each of 200 r (200 r skin dose by 140 KVP, 15 ma. 3.02 min., at 70 cm. with no filter). The exposures were limited to the head and were spaced seven days apart with the carcinogen injected either (a) three or four days before the first exposure, (b) between the exposures, or (c) two to four days following the final irradiation. Controls for this group were not operated but were given 400 to 600 r under the same protocol (fig. 3).

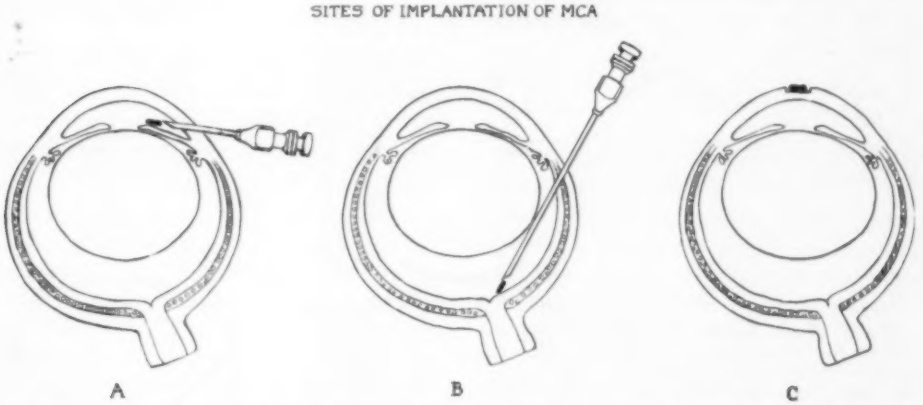

Fig. 2 (Patz, Wulff, and Rogers). Schematic diagram showing methods of introducing methylcholanthrene into the eye with a hypodermic needle. (a) Packed crystals are injected into the anterior chamber. (b) Needle is introduced into the vitreous. (c) Corneal epithelium is abraded, MCA applied, and lids are sutured.

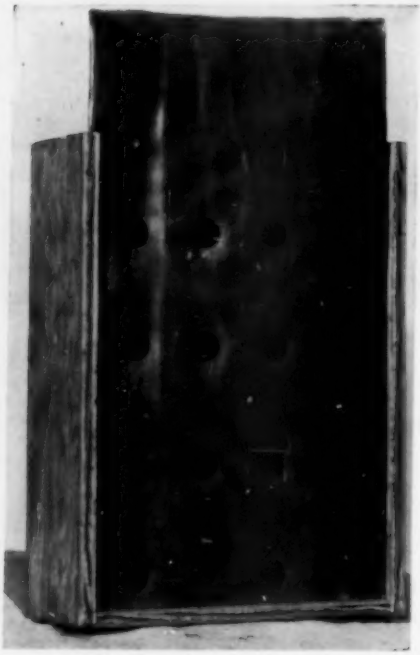

Fig. 3 (Patz, Wulff, and Rogers). Lead shielded container protects body of mice while head is exposed to X-radiation.

One group of animals operated five to six months previously and showing some intraocular changes that were not progressive in a three-month period were also irradiated as above.

3. SUBCUTANEOUS IMPLANTS (C_{57}, A, A/Jax, CF_1)

Whole eyes, uvea, or retina were apposed to fused MCA crystals and placed subcutaneously in groin or axilla. The donor animal was also recipient.

After making an incision in the abdominal wall large enough to accommodate a fine forceps the eye was excised or dissected, MCA placed in the organ, the implant then located subcutaneously with smooth forceps, and the wall sutured. Implants in newborns were placed in the scruff of the neck.

Efforts to locate and fix tissue in the anterior chamber of the mouse eye were notably unsuccessful.

Retinas dissected from the eyes of 19-day fetuses, newborn, and mature mice were cultured in vitro as viability controls for the mechanics of the implant experiment. Whole eyes and segments were also planted subcutaneously without MCA as controls.

4. SUTURE IMPLANTATION (C_3H)

Sutures impregnated with 20-methylcholanthrene and cholesterol were drawn through and fixed within anterior chamber (including iris margin), posterior segment, harderian gland or sclera of C_3H mice of both sexes, six to eight weeks old (fig. 4). Forty-one infant C_3H mice were operated similarly, but the suture of necessity was located less specifically and the entire deep orbital contents were included in the suture bed.

Preparation of the suture. 6-0 black silk sutures with atraumatic needles were first soaked in chloroform. When the chloroform had evaporated the sutures were placed in a molten mixture of 20-methylcholanthrene

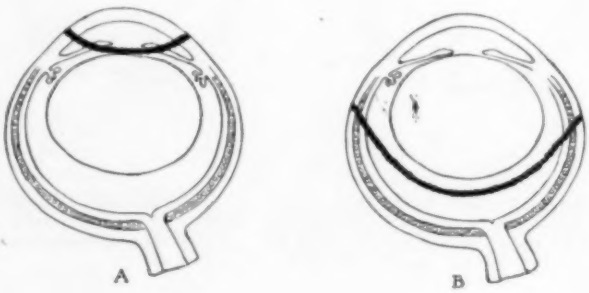

Fig. 4 (Patz, Wulff, and Rogers). Schematic position of MCA-impregnated suture introduced into the anterior chamber and through vitreous.

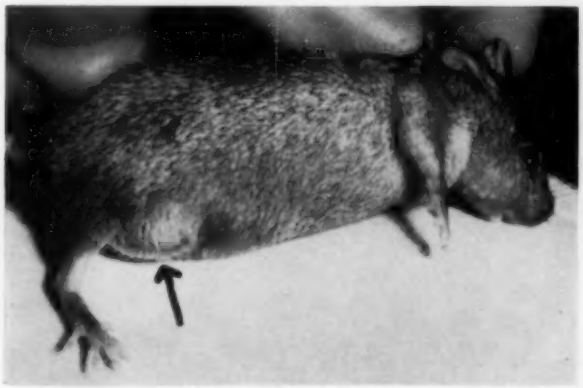

Fig. 5 (Patz, Wulff, and Rogers). Arrow points to squamous cell carcinoma transplant. A one-mm. piece of tumor from eye of Animal 79-2 was transplanted subcutaneously one month prior to photograph.

and cholesterol, 1:3. After five minutes the sutures were withdrawn and straightened to dry. Needles were cleaned of crystalline deposit by dipping in chloroform. Large deposits of the yellow mixture could readily be seen along parts of the suture in ordinary white light. Ultraviolet lamp examination (Woods lamp) showed fine, even deposition the length of the suture.

Operating procedure. The animals were immobilized manually and the eye proptosed. The globe was fixed with forceps and the operator threaded the suture in position, clipped each end close to the point of penetration, and the adnexa were cleaned of any scattered crystals with a moistened swab. In many instances an area of suture containing a large precipitate of carcinogen was introduced.

Transplants. Seven of the eyes that presented gross enlargement in some part of the organ within the experimental period were transplanted. Sections of the growth were taken when it had seemed to reach the maximum possible size without rupturing the globe and transplanted. For this transplant the donor animal was anesthetized by sodium pentobarbitol intraperitoneally. Recipient animals, 14 to 30 days old, were not anesthetized nor pretreated in any way. Transplants were made subcutaneously over the right shoulder or into the right groin.

Those transplants that showed active growth were followed periodically and when their growth reached about one cm. in diameter the capsule was opened and sections of the tumor were taken for histologic study for transplanting a second time (fig. 5).

Animals that had not been killed earlier because of gross ocular defects or systemic ailments were killed 6.0 to 10.5 months postoperatively and the heads and other organs which appeared abnormal were fixed in Bouin's solution. Serial sections of the anterior cranium were cut at 6.0 microns and stained routinely with hematoxylin-eosin. Special stains were employed when abnormalities were detected in histologic study.

Results

A. General observations

Within the first 48 hours all the operated animals showed a varying degree of conjunctival hyperemia and swelling of the lids. In the majority of the animals injected with either packed MCA crystals or pellets this conjunctival hyperemia persisted for about seven days, then gradually cleared. In the suture operated eyes the reaction was more severe and infection more frequent. In approximately one fifth of the eyes injected with plain crystals, the inflammatory reaction increased, the cornea became diffusely cloudy, and the reaction progressed to early phthisis bulbi at the end of the second week. Phthisis bulbi occurred in about one-half of the suture

TABLE 1

TABLE OF OPERATIVE PROCEDURES

Procedure	Strain	Number of Eyes				
		Anterior Chamber	Posterior Segment	Extraocular	Cornea	Others
Injection	A ♂	12*	66*			
	A ♂	26	38			
	A/Jax	34	34*		6	
	CF ♀	6	20		6	
	C_{57}	3				
Controls	A ♂, CF ♀		22			
Injection+X rays	A/Jax ♀	38	48			
Controls	A/Jax ♀					34
Implants	C_{57}					8
	A ♂					7
	CF, ♀					5
Controls	C_{57}, A/Jax					14
Suture	C_3H (NIH)	14	38			
	C_3H (CF)		26	12	16 (incl. sclera)	
Controls	C_3H (CF)	8	8	6		
Suture (newborn)	C_3H (NIH)					41

* These eyes were denuded before injection.

operations. The operated eyes that withstood the operation and did not go to phthisis remained essentially quiescent after the second week, at which time there was usually some degree of cataract formation visible. Even in those eyes that subsequently developed tumors, little change was observed from the second week until about three months after operation. When the eyes were examined under a Woods fluorescent light it was noted that the MCA-crystals frequently showed active fluorescence up to two months after operation. However, residual fluorescence from the crystals could not be detected after this period.

In histologic examination of the eyes that did not develop malignant tumors there was evidence of chronic inflammatory reaction with varying degrees of necrotic and atrophic changes. In some of the more severely involved eyes the entire retina and uvea were replaced by vascularized fibrous tissue. In others there were only mild atrophic and chronic inflammatory changes in the general area where the MCA was implanted. Where marked necrosis of adjacent structures occurred it was interesting to note that the pigment epithelium frequently persisted in a distinct layer of cells. In some instances the pigment epithelium lined cystic spaces filled with blood or exudate. Varying degrees of cataractous changes were noted in the majority of operated eyes. This, however, was anticipated in view of the relatively large size

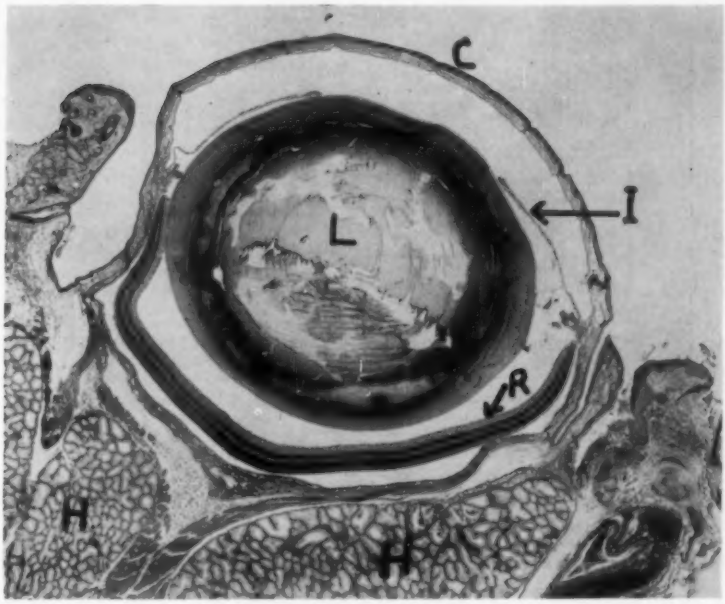

Fig. 6 (Patz, Wulff, and Rogers). Cross section of normal mouse eye. Note relative size of lens and other intraocular structures. (L–lens, I–iris, R–retina, H–harderian gland, C–cornea.)

of the lens which fills the bulk of the globe (fig. 6). This was inadvertently traumatized at the time of operation.

No ocular tumors developed in any of the controls to these four experiments. Most of the controls from Experiment 1 showed only a limbal scar or scar through the posterior segment at site of needle penetration which could be located in serial section. An occasional eye showed detachment and gliosis of the retina. Some of the animals showed small zones of cataractous changes in the lens. The X-ray control animals showed cataract formation histologically but were otherwise normal. The control retinas that were transplanted subcutaneously could not be located in serial section and no tumors developed in the MCA treated retinas that were transplanted.

B. HISTOLOGIC DETAILS OF INDUCED TUMORS

1. *Corneal epithelial tumor* (Animal No. 20-6). The tumor originates in the corneal epithelium and infiltrates the entire thickness of the cornea. Mitoses are numerous and there are chronic inflammatory cells at the base of the tumor. The cells show prominent nucleoli, some hyperchromatism, and a moderate degree of pleomorphism. Near the surface of the tumor some cells take a more eosinophilic stain and have lost nuclear detail to resemble slightly the epithelial pearl appearance characteristic of squamous cell carcinoma. No typical pearls are seen. However, since pearl formations indicate a certain degree of epithelial differentiation they are not usually seen[3] in a highly malignant rapidly growing tumor of this type (figs. 7 and 8).

HISTOPATHOLOGIC IMPRESSION: Squamous-cell carcinoma, Grade III, of the corneal epithelium.

2. *Conjunctival epithelial tumors* (Animals No. 79-2, 74-5, 73-3, 13465). In all of these specimens the conjunctival tumors invaded the globe and in most cases filled the

TABLE 2

TABLE OF INDUCED TUMORS

Animal	Strain	Classification	Procedure
20-6	A ♂	Squamous cell carcinoma of cornea	MCA applied to cornea and lids sutured
79-2	C_3H (CF)	Squamous cell carcinoma of conjunctiva	6-0 suture with 25 percent MCA in cholesterol posterior segment
74-5	C_3H (NIH)	Squamous cell carcinoma of conjunctiva	6-0 suture with 25 percent MCA in cholesterol in posterior segment. Repeated 5½ months later
13451	A (Md)	Squamous cell carcinoma of conjunctiva	MCA injected in anterior chamber
73-3	C_3H (NIH)	Squamous cell carcinoma of conjunctiva	6-0 suture with 10 percent MCA in cholesterol in posterior segment
13485	A (Md)	Squamous cell carcinoma developing in epithelial downgrowth	MCA injected in anterior chamber
13482	A (Md)	Squamous-cell carcinoma developing in epithelial downgrowth	MCA injected in anterior chamber
13463	A (Md)	Adenocarcinoma of harderian gland	MCA injected in posterior segment
72-6	C_3H (NIH)	Adenocarcinoma of harderian gland	6-0 suture with 10 percent MCA—cholesterol through posterior segment
73-4	C_3H (NIH)	Adenocarcinoma of harderian gland	6-0 suture with 10 percent MCA—cholesterol through posterior segment
48-2	A/Jax	Malignant tumor of iris unclassified	MCA injected anterior chamber followed by 200 r X rays two times
12-11	CF_1	Malignant tumor of iris unclassified	MCA injected one mm. postlimbal
20-x	A/Jax	Malignant tumor of iris unclassified	MCA crystals injected anterior chamber
74-2	C_3H (NIH)	Malignant melanoma of choroid	6-0 suture with 10 percent MCA—cholesterol in posterior segment. Repeated 5½ months later
72-4	C_3H (NIH)	Malignant melanoma of choroid	6-0 suture with 10 percent MCA in cholesterol in posterior segment
73-6	C_3H (NIH)	Malignant melanoma of choroid	6-0 suture with 10 percent MCA in cholesterol in posterior segment
42-4	A/Jax	Sarcoma of orbit	MCA crystals in posterior segment

greater portion. Their histologic appearance is essentially similar. The cells are prickle-cell in appearance and the cytoplasm is acidophilic. In some areas the cells have lost their nuclear detail and abortive epithelial pearl formation is noted. Mitoses are numerous. The tumors are relatively well differentiated and show only mild pleomorphism. There is a mild chronic inflammatory reaction about the surface of the tumors (figs. 9, 10, and 11).

HISTOPATHOLOGIC IMPRESSION: Squamous-cell carcinomas, Grades II-III, of the conjunctival epithelium with invasion of the globe.

3. *Tumors in epithelial downgrowths* (Animals No. 13482, 13485). The development of squamous-cell carcinoma in downgrowth

TABLE 3

TABLE OF TRANSPLANTED TUMORS

Animal	Site of Suture	Time (mo. operation to transplant)	Transplant	Take	2nd Transplant	Description
79-2	Fundus	3½	Yes	Yes	Yes	Squamous-cell carcinoma of conjunctiva
74-5	Fundus	7½*	Yes	No	—	Squamous-cell carcinoma of conjunctiva
74-2	Fundus	7½	Yes	No	—	Melanoma of choroid
73-4	Fundus	7*	Yes	No	—	Harderian gland adenocarcinoma
73-3	Fundus	7½	No	No	—	Squamous-cell carcinoma of conjunctiva
72-4	Fundus	3½	Yes	No	—	Melanoma of choroid
73-6	Fundus	3	Yes	No	—	Melanoma of choroid

* These animals were operated twice, the first suture being placed seven months prior to appearance of the tumor and the second operation two months previously.

of surface epithelium into the anterior chamber occurred in two eyes. These showed a relatively low grade of malignancy and were all essentially similar. Dense sheets of a keratinlike mass are abundant in and about the tumor cells. The cytoplasm is granular and many of the cells are eosinophilic and have the prickle-cell appearance characteristic of squamous-cell carcinoma. Occasional mitoses are present (fig. 12).

HISTOPATHOLOGIC IMPRESSION: Squamous-cell carcinomas, Grade I, developing in surface epithelial downgrowth into the anterior chamber.

4. *Harderian gland tumors* (Animals No. 13463, 73-4, 72-6, and 13482). The adenocarcinomas of the harderian gland are all similar and show a typical abortive glandular appearance. In some of the tumors large hemorrhages were found. Nuclear hyperchroma-

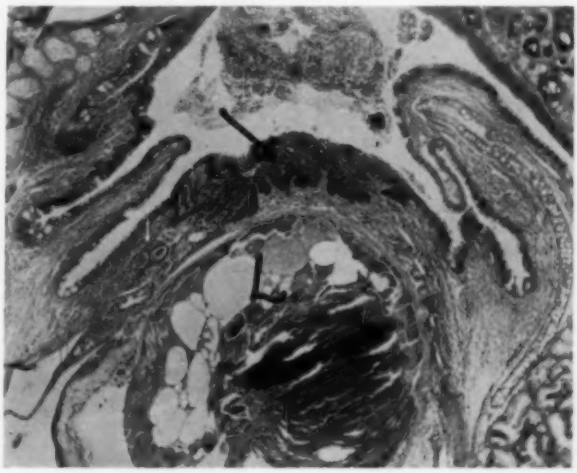

Fig. 7 (Patz, Wulff, and Rogers). Arrow points to squamous cell carcinoma of cornea infiltrating corneal stroma. (L- cataractous lens, (Hematoxylin-eosin X36, Animal 20-6.)

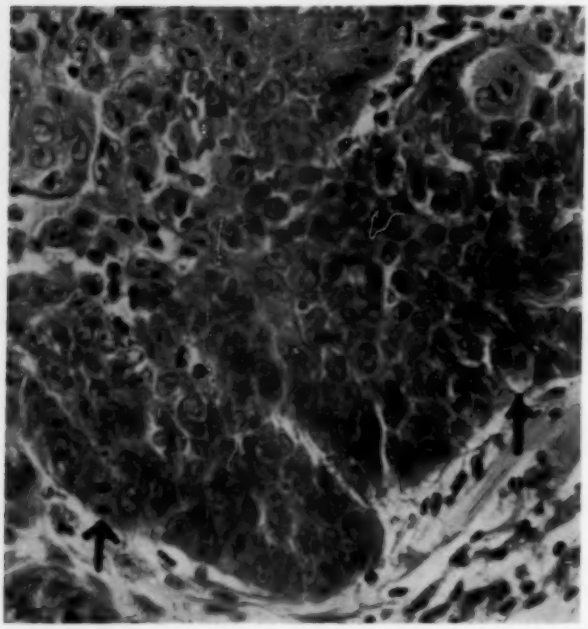

Fig. 8 (Patz, Wulff, and Rogers). High-power view of corneal tumor in Figure 7. Arrows point to mitotic figures. Note pleomorphism and moderate degree of anaplasia. (Hematoxylin-eosin X500, Animal 20-6.)

tism and prominent nucleoli are present as well as numerous mitoses in all of the tumors. In some areas of the tumors the alveoli contain an amorphous hyaline-like coagulum (figs. 13 and 14).

HISTOPATHOLOGIC IMPRESSION: Adenocarcinoma of harderian gland.

5. *Iris tumors* (Animal No. 48-2, 12-11, and 20-x). The three iris tumors closely resemble each other and show essentially the

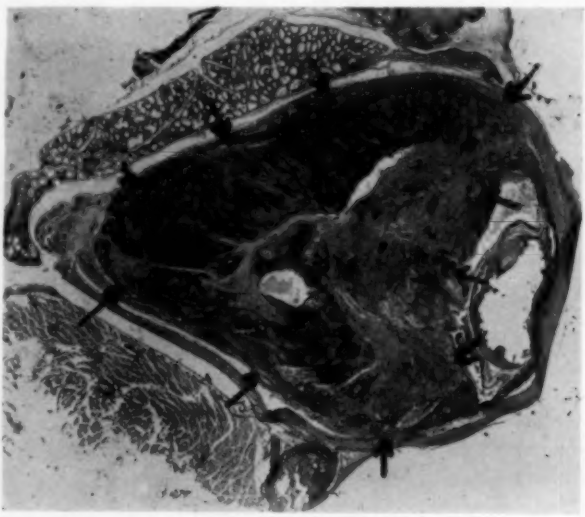

Fig. 9 (Patz, Wulff, and Rogers). Squamous-cell carcinoma filling entire globe and invading orbit. In serial sections tumor found to originate from conjunctival epithelium. Arrows delineate tumor extension. (Hematoxylin-eosin X30, Animal 79-2.)

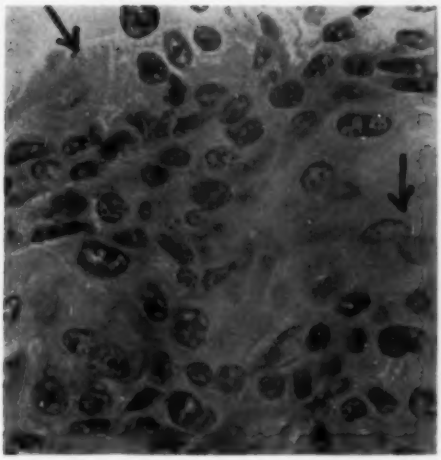

Fig. 10 (Patz, Wulff, and Rogers). High-power view of tumor in Figure 9. Note moderate degree of anaplasia and hyperchromatic nuclei. Arrows point to cells with prickle-cell characteristics. (Hematoxylin-eosin X500, Animal 79-2.)

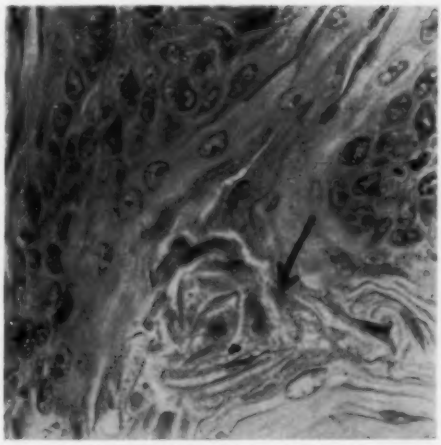

Fig. 11 (Patz, Wulff, and Rogers). Section through area of transplanted tumor seen in Figures 5 and 10. Note similarity to original tumor. K-keratin like material being deposited in tumor. (Hematoxylin-eosin X500, Animal 79-2 transplant 1.)

same cellular architecture. The cells are densely packed and infiltrate the entire thickness of the iris. There is a fine network of vessels on the anterior surface of the tumors. The cells are relatively uniform in appearance. The cell walls are indistinct. Some cells show hyperchromatic nuclei and prominent nucleoli. The nuclei are slightly ovoid for the most part. There is a fine reticulum sparsely spread out in the deeper portions of the tumor near the posterior part of the iris.

Although these tumors closely resemble human malignant melanoma of the iris they are not identical histologically to any specific type of human melanoma. The tumors all developed in albino mice. No pigment is

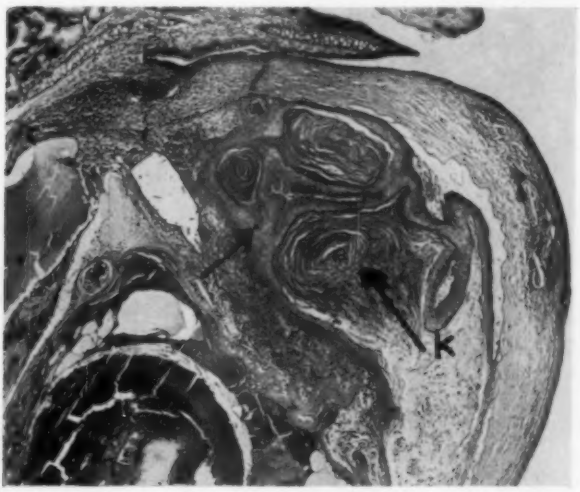

Fig. 12 (Patz, Wulff, and Rogers). MCA-operated eye where hypodermic needle containing pellet was introduced through limbus into anterior chamber. Surface epithelial invasion of the anterior chamber occurred and low grade squamous cell carcinoma is developing in ingrown epithelium. Arrow points to area of early squamous cells carcinoma formation. K–Keratinlike sheets piled up adjacent to proliferating epithelium. (Hematoxylin-eosin X50, Animal 13485-15).

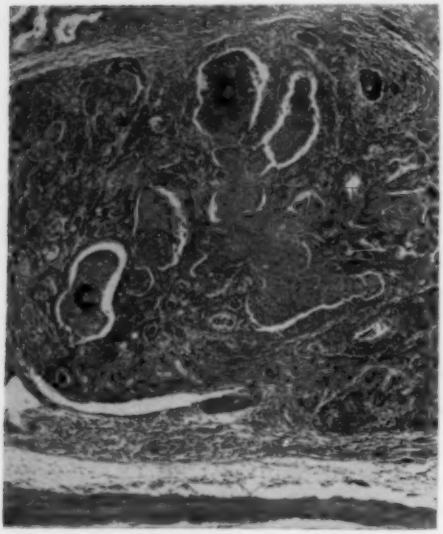

Fig. 13 (Patz, Wulff, and Rogers). Adenocarcinoma of harderian gland. Note glandular arrangement of tumor and large area of amorphous coagulum in tumor. (Hematoxylin-eosin X50, Animal 13463151.)

present in these tumors (figs. 15 and 16).

HISTOPATHOLOGIC IMPRESSION: Malignant tumors of the iris, type as yet unclassified.

6. *Choroidal tumors* (Animals No. 74-2, 72-4 and 73-6). The three choroidal tumors which are classified as probable malignant melanomas have slight variations in histologic detail and are described individually. The choroidal tumors all developed in C_3H mice, a moderately pigmented strain.

A. Animal 74-2. The tumor has filled the entire globe. Only small remnants of lens cortex are seen in serial sections and the lens capsule is folded and surrounded by tumor. The corneal stroma is infiltrated with tumor and chronic inflammatory cells. The corneal epithelium is absent centrally and has undergone hyperplasia peripherally. The iris and ciliary body are atrophic and are densely infiltrated with tumor cells. The retina has degenerated except for a few small segments that are detected in serial sections. The choroid is infiltrated with tumor. The sclera is intact except posteriorly where in serial section the apparent site of rupture of the globe with spread of tumor into the orbit is seen. There is diffuse infiltration of the entire orbit with tumor which invades the harderian gland and extraocular muscles.

The tumor mass is densely packed with

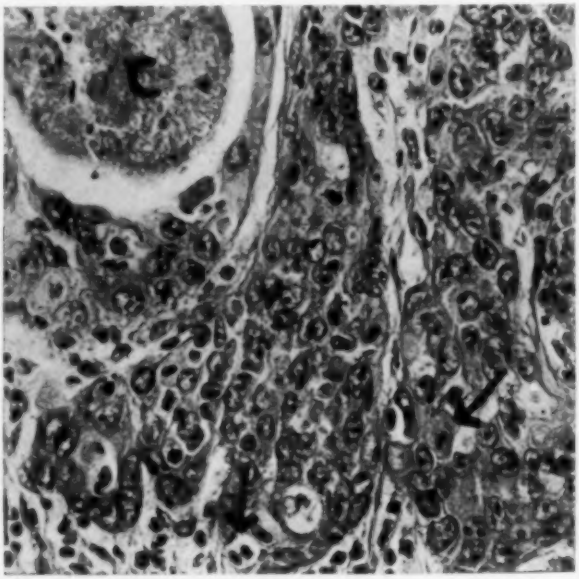

Fig. 14 (Patz, Wulff, and Rogers). Higher power of adenocarcinoma in Figure 13. Arrows point to mitotic figures. C–amorphous coagulum in lumen of gland. Note typical glandular arrangement of tumor. (Hematoxylin-eosin X500, Animal 13463-51.)

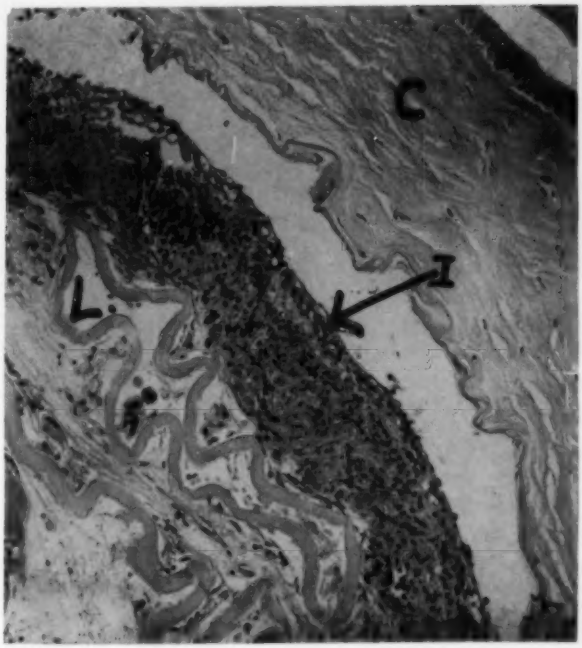

Fig. 15 (Patz, Wulff, and Rogers). Section of anterior chamber of eye where MCA pellet was introduced into anterior chamber on surface of iris and animal subsequently irradiated. Note diffuse invasion of entire iris stroma with tumor cells. C–cornea, I–iris, L–lens capsule. (Hematoxylin-eosin X150, Animal 48-2-12.)

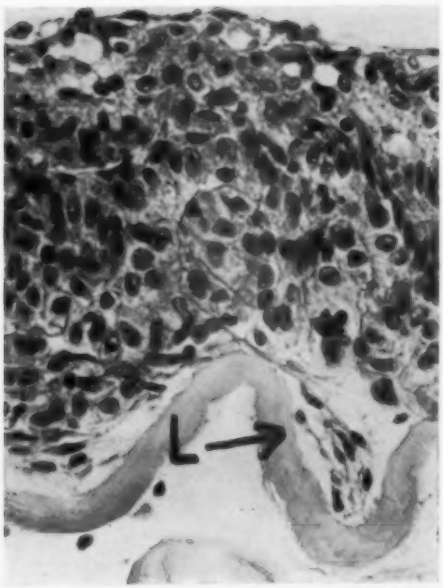

Fig. 16 (Patz, Wulff, and Rogers). High-power view of Figure 15 showing tumor cells infiltrating entire iris structure. L–lens capsule. (Hematoxylin-eosin X500, Animal 48-2-12.)

tumor cells and chronic inflammatory cells consisting of lymphocytes and plasma cells. The tumor is relatively avascular with a few fine vessels noted throughout the tumor. There are no areas of necrosis within the tumor proper. There is a fine sparse reticular network best seen with Wilders reticulum stain. The tumor cells are round or ovoid with moderate variation in size and shape. There are occasional multinucleated and bizarre cell forms. The nuclei are large and round and prominently nucleolated. The cytoplasm is abundant, homogenous, and acidophilic. There are scattered clumps of pigment apparently carried into the tumor from degenerated melanocytes of the iris and choroid in the immediate uveal area. There are, in addition, several areas near the center of the tumor and in the orbital portion which are well away from uveal tissue where fine pigment granules have apparently been liberated by the tumor cells themselves. In one area of the choroid the tumor cells take a definite spindle form. Mitoses are numer-

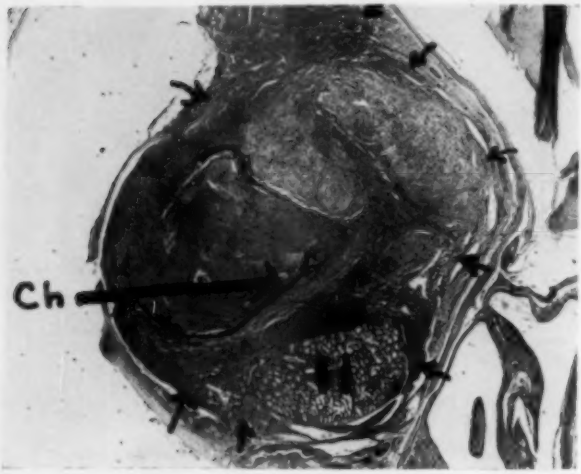

Fig. 17 (Patz, Wulff, and Rogers). Low-power view of tumor apparently originating in choroid having erupted through the globe and filling entire orbit displacing globe. Ch–choroid, H–harderin gland. Arrows show tumor extension into entire orbit. (Hematoxylin-eosin, X36, Animal 74-2.)

ous. In serial sections the remains of the silk suture can be traced through the posterior segment and passing through the choroid. The exact site of origin of the tumor along the suture tract could not be determined (figs. 17, 18, and 19).

HISTOPATHOLOGIC IMPRESSION: Malignant melanoma, epitheloid type, apparently originating from choroid with rupture of globe and invasion of orbit.

B. Animal 72-4. The tumor fills the posterior segment and has spread in the region of the optic nerve through the sclera to fill the entire orbit. The extraocular muscle fibers are separated by tumor cells. The globe is markedly proptosed. The cornea is atrophic and centrally there is an area of apparently recent perforation. This zone of the cornea and the entire anterior chamber is filled with polymorphonuclear leukocytes. There is old hemorrhage in the anterior chamber with many hemosiderin pigment laden macrophages present. The iris and ciliary body are atrophic and densely infiltrated with tumor cells. The lens capsule is intact except for one area posteriorly where tumor cells pene-

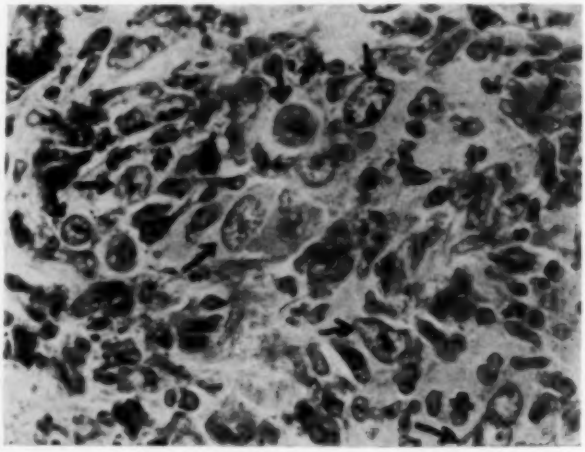

Fig. 18 (Patz, Wulff, and Rogers). High-power view of tumor shown in Figure 17. Arrows point to several epithelioid-like cells showing large oval nuclei with prominent nucleoli and scanty cytoplasm. Note marked anaplasia. (Hematoxylin-eosin, X500, Animal 74-2.)

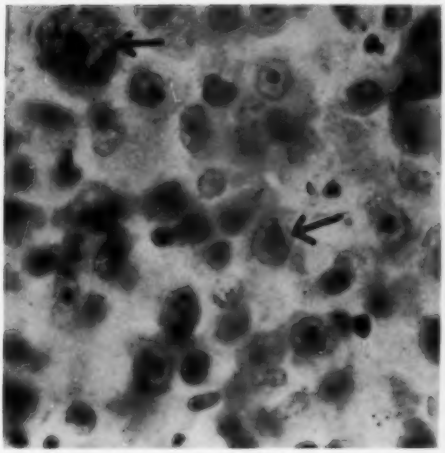

Fig. 19 (Patz, Wulff, and Rogers). Section of human malignant melanoma of choroid, epithelioid type. Note similarity to animal tumor in Figure 18. Arrows point to several cells showing large oval nuclei, prominent nucleoli, and scanty cytoplasm. Note similarity to mouse tumor in Figure 18. (Hematoxylin-eosin, X500.)

trate it for a short distance. The lens cortex has disappeared and is replaced by a coagulum of polymorphonuclear leukocytes, hemosiderin laden phagocytes, and an amorphous eosinophilic material. The lens nucleus is normal in appearance, but displaced. The choroid is infiltrated with tumor and the retina has completely disappeared. The sclera is intact except at the site of perforation posteriorly by the tumor.

The tumor is densely packed with tumor cells with an occasional chronic inflammatory cell interspersed. The cells are long oval in shape with a scanty acidophilic cytoplasm. The nuclei are prominently nucleolated. Mitoses are abundant. In some areas the long oval tumor cells are arranged in sheets in a palisaded arrangement. There is no pigment liberation in the tumor although degenerated melanocytes from the uveal tract are present in the areas immediately adjacent. There is only a sparse, very fine reticular network present. The silk suture tract is readily traced through the posterior segment in serial section (figs. 20, 21, and 22).

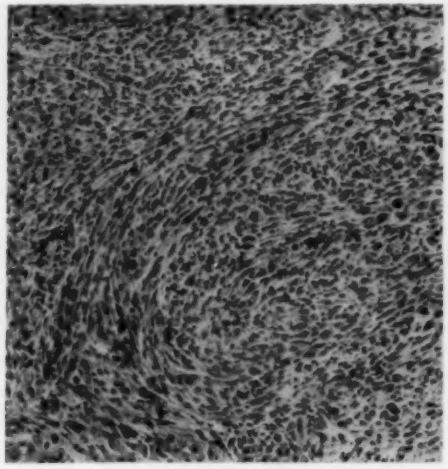

Fig. 20 (Patz, Wulff, and Rogers). Intermediate-power view of mouse choroidal melanoma showing fascicular arrangement of bundles of spindle cells. (Hematoxylin-eosin, X100, Animal 72-4.)

HISTOPATHOLOGIC IMPRESSION: Malignant melanoma, Spindle B type, apparently originating from choroid with invasion of orbit.

C. Animal 73-6. The tumor fills the globe

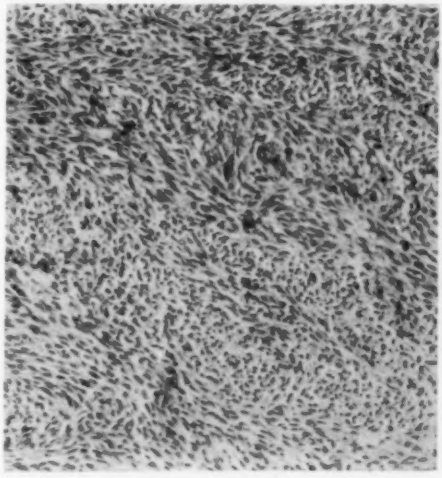

Fig. 21 (Patz, Wulff, and Rogers). Intermediate-power view of human malignant melanoma, spindle B type with fascicular arrangement of cells. Note similarity to mouse tumor in Figure 20. (Hematoxylin-eosin, X100.)

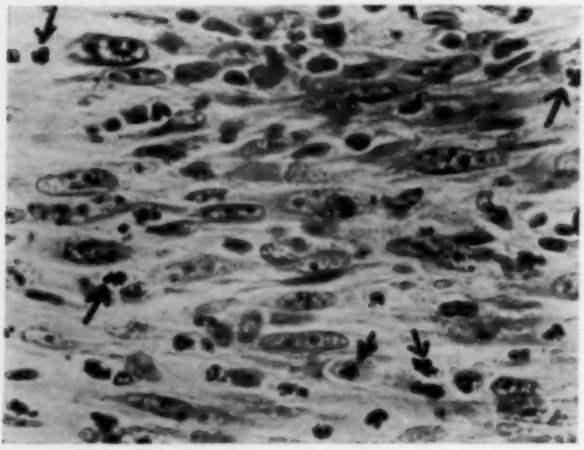

Fig. 22 (Patz, Wulff, and Rogers). Higher power view of mouse choroidal melanoma seen in Figure 20. Note hyperchromatic oval nuclei with prominent nucleoli. Arrows point to a few of the mitotic figures present. (Hematoxylin-eosin, X500, Animal 72-4.)

and has ruptured it posteriorly to fill most of the orbit. Some portions of the harderian gland are not infiltrated with tumor and appear normal. The globe is markedly proptosed and the cornea has perforated. The anterior chamber is filled with a coagulum of serum, polymorphonuclear leukocytes, hemosiderin laden phagocytes, and degenerated iris pigment. The iris and ciliary body are completely infiltrated by tumor and no normal iris landmarks remain. The lens is invaded by tumor cells and only a small portion of the nucleus remains. The choroid is diffusely infiltrated by tumor. The sclera is atrophic and there is a large defect posteriorly where the tumor invades the orbit. The retina has disappeared except for a few small remnants posteriorly.

The tumor consists of predominantly spindle-shaped cells with a narrow oval nucleus and scanty acidophilic cystoplasm. In

Fig. 23 (Patz, Wulff, and Rogers). Choroidal melanoma invading right orbit with proptosis resulting. Arrows delineate tumor extension. MCA suture implanted in globe three months previously. (Animal 73-6.)

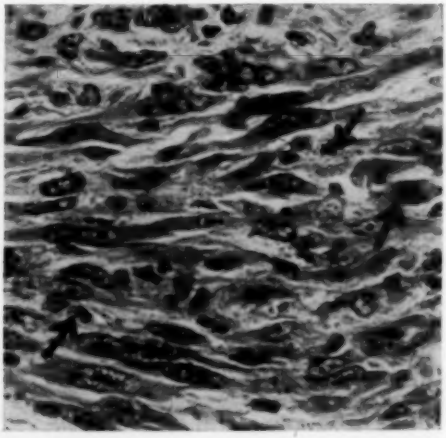

Fig. 24 (Patz, Wulff, and Rogers). High-power view of section of choroidal melanoma observed grossly in Figure 23. Spindle cells are narrower and nucleoli are less pronounced when compared to tumor in Figure 22. Arrows point to mitotic figures. (Hematoxylin-eosin, X500, Animal 73-6.)

Fig. 25 (Patz, Wulff, and Rogers). High-power view of another area of tumor observed in Figure 24. The nuclei here are more oval, less elongated, and show prominent nucleoli. Arrows point to several of the numerous mitotic figures. (Hematoxylin-eosin, X500, Animal 73-6.)

an occasional cell a line or fold extends through the center of the nucleus longitudinally. The nucleoli are generally less pronounced than in the previous two choroidal tumors. In a few areas the nuclei are more oval and are prominently nucleolated. Occasional lymphocytes and polymorphonuclear leukocytes are scattered through the tumor. There is a fine well-defined reticular network throughout the tumor. The tumor is poorly vascularized with a network of fine vessels. There are no pigment cells in the tumor proper except in the areas where the uveal tract is invaded. There are numerous mitotic figures especially in the extraocular part of the tumor (figs. 23, 24, and 25).

HISTOPATHOLOGIC IMPRESSION: Malignant melanoma, predominantly Spindle A type, apparently choroidal origin with invasion of orbit.

7. Sarcoma of orbit. The tumor fills the entire orbit and no remains of the globe are found in section. The tumor is sparsely vascularized with a network of fine capillaries. Most of the cells are fusiform in appearance and there is a prominent fibrillary stroma. The nuclei are narrow and elongated resembling fibroblasts in many areas. Some cells are more oval and prominently nucleolated, however. Mitotic figures are numerous (fig. 26).

HISTOPATHOLOGIC IMPRESSION: Fibrosarcoma of orbit.

DISCUSSION

Before discussing the data collected in these experiments a brief résumé of some observations that are fundamental to experimental carcinogenesis are cited. The precise mechanism whereby chemical carcinogens induce malignant change in normal cells probably is as obscure as is the development of clinical cancer itself. The process of experimental carcinogenesis, however, can be divided into an initial phase of irritation due to the toxic action of the chemical, followed by a latent period of several months, then the period of actual neoplasia.[3-5]

The experimental findings of many investigators, notably those of Berenblum and Shubik[6] and Orr,[7] on the effects of carcinogenic hydrocarbons applied to the skin, show initially a typical response to a local irritant. During the first two days there is excessive proliferation of epithelial cells and increased keratin formation. Cytoplasmic degeneration

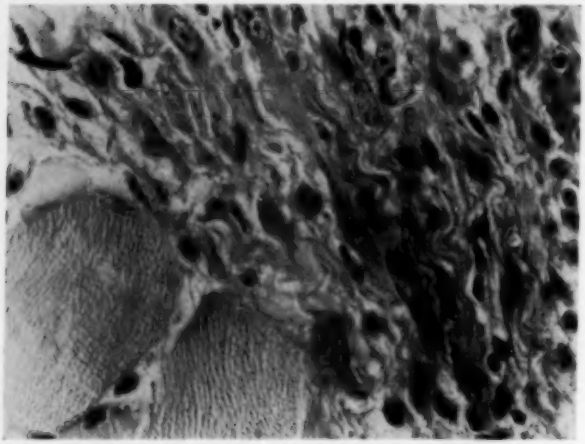

Fig. 26 (Patz, Wulff, and Rogers). High-power view of orbital sarcoma. Note bundles of collagenous fibers. Some nuclei are long and thin, others more oval and prominently nucleolated. (Hematoxylin-eosin X500, Animal 42-4.)

and breakdown of the epithelial cells occurs about the third day with an increase in the subepithelial connective tissue and local congestion of the blood vessels. About the fourth day, tissue repair begins and there is a gradual return to a normal appearance of the skin. After a latent period of three months or longer, the process of true neoplasia occurs. The cells become irregular in size, mitoses are prominent, the arrangement of cells becomes irregular. Continued proliferation occurs with the development of carcinoma.

Cramer and Simpson, 1945,[8] noted that following the skin application of a single *small* dose of carcinogen, inadequate to produce tumor, the initial effect of local irritation occurred but, following the usual latent period, no tumors developed. However, if following the single application of this same small amount of carcinogen the non-specific effect of burning the skin was added, tumors developed at the burn site. Deelman, 1924,[9] showed that tumors occurred at the edge of a deep skin wound inflicted following the application of a *small* dose of carcinogen that was normally insufficient to produce tumors. Yet repeated application of injury or non-specific irritants alone do not produce tumors. These observations together with their own similar data led Berenblum and Shubik[6] to conclude that the initial irritant action or "initiating action" is a specific one and is only produced by a definite carcinogen. Once the "initiating action" produced by a specific carcinogen has occurred in the tissue, then a non-specific irritant, for example, heat or trauma, may exert a "promoting action" and neoplasia follows. They reasoned that the specific carcinogenic chemical changes a few normal cells into latent tumor cells (initiating action) on a single application. If the initial irritant effect is potent enough, tumors will develop without a secondary promoter. If the initial carcinogen is applied in a very small amount, only the initiating effect occurs. Either a repetition of the carcinogen or the application of a nonspecific irritant or "promoter" will then be required to induce neoplasia. These so-called promoters are usually designated co-carcinogens.

The co-carcinogens, though not themselves necessarily carcinogenic, may also act during the initial application of the carcinogen.[3] Their effect can be (1) to shorten the latent period of actual neoplasia where the initial carcinogen is sufficiently potent to produce tumors and (2) promote the production of tumor where the carcinogen dosage itself is insufficient. X-radiation to the head was utilized in Experiment 2 already cited as a promoter following MCA treatment. Certain

solvents which enhance the transfer of the specific carcinogen across the cell wall can also enhance the tumor yield. Cholesterol, which raises the rate of transfer of the carcinogen, was used here in Experiment 4 for this purpose.

It is known that 20-methylcholanthrene is a potent cancer hydrocarbon and acts as its own "initiator" and "promoter" when in sufficient concentration.[3] Methylcholanthrene is of special interest not only because of its potent experimental carcinogenic effect, but also because it is closely related chemically to the naturally occurring bile acids in the human and animal species. Methylcholanthrene can be prepared from both desoxycholic acid and cholic acid. Figure 27 shows the structural formula of methylcholanthrene.

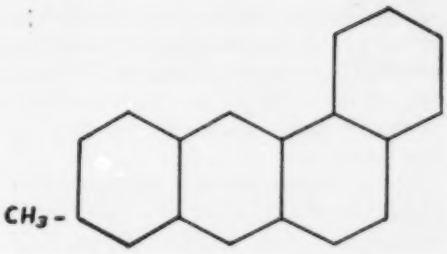

Fig. 27 (Patz, Wulff, and Rogers). Structural formula of methylcholanthrene.

Previous ocular tumor studies

Two of the seven types of tumors observed have been previously reported, namely harderian gland carcinoma and orbital sarcoma. Harderian gland tumors have been noted by Benedict[10] following total body irradiation of mice and they have occurred following oral administration of acetyl aminofluorine.[11]

Gondolphi and Tanzi, 1941,[12] reported fuso-cellular sarcoma in rat eyes treated with 3-benzpyrene in olive oil. Thomas, 1937,[13] has utilized methylcholanthrene in an unsuccessful attempt to induce ocular tumors. Scarlet red[14,15] and indole[16] have also been injected without success. Weil and Mayer[17] reported dibenzanthracene in lard solution ineffectual, but their control rats injected with lard not containing the carcinogen showed changes in the retina which they termed "retinoblastoma." Their photomicrographs appear to show simply the massive gliosis and folding of the retinas that frequently occur clinically where a patient sustains a posterior segment penetrating wound. Several of our control animals operated by inserting a plain hypodermic needle into the vitreous showed retinal gliosis and proliferative changes essentially similar to their reported observations.

The incidence of infection and degeneration of the globe was much higher in the animals operated by inserting an MCA-impregnated suture. This technique, however, permitted a much more accurate and predictable placement of the carcinogen. In current studies the suture insertion is being used virtually exclusively. Instead of impregnating the entire suture length with carcinogen a small bead of molten MCA is applied to the suture.

The failure to produce retinal tumors may have been due to the improper placement of the carcinogen in the posterior segment. One other explanation may lie in the simultaneous stimulation of both retinal and choroidal cells by the MCA. The latent period for tumor formation from the choroid may be shorter than for retinal tumor formation. As a result the choroidal tumors which appeared to grow rapidly may have destroyed any latent retinal tumor cells. In current studies an attempt is being made to apply the carcinogen to the internal surface of the detached mouse retina to avoid simultaneous stimulation of choroidal elements.

The principal aim of the retinal carcinogenesis experiments is to produce retinoblastomas that may then be experimentally treated to evaluate therapy. The unique response of the premature retina to hyperoxia[18] suggested to us that short periods of oxygen inhalation combined with X-irradiation might augment the effect of radiation on

retinoblastomas. This concept is based on the evidence of Gerschman and co-workers[19] that the effect of oxygen poisoning and X-irradiation are cumulative in the tissues and that a possible common mechanism for their actions exists.

The crystalline lens was frequently traumatized by the operative procedure, but no attempt was made to introduce the MCA pellets or sutures within the lens capsules to stimulate tumor formation from the lens epithelium. Mann[20] has previously demonstrated the malignant potentialities of lens epithelium experimentally by tissue culture and subcutaneous transplant techniques.

Relationship of mouse tumors to clinical neoplasms

The squamous cell carcinoma of the corneal epithelium diffusely infiltrated the corneal stroma. This tumor may be analogous to the squamous-cell changes that Reese[21] has cited can occur in Bowen's disease of the cornea. The mouse tumor was a rapidly growing moderately anaplastic squamous cell carcinoma.

The conjunctival squamous cell carcinomas were markedly invasive and penetrated the globe to displace and replace the normal intraocular structures. One of these tumors has been transferred in subcutaneous transplants two times to date and the subcutaneous tumors grow readily (fig. 5). The tumor has the characteristics of typical mucous membrane carcinoma and is probably analogous clinically to the squamous-cell carcinomas of the limbus or conjunctiva.

The carcinomas that developed in the mouse epithelial downgrowth are not analogous to any clinical cases since carcinoma formation in postcataract extraction epithelial downgrowth has not been reported. One case of marked epithelial proliferative activity in clinical downgrowth has been observed, however.[21]

The mouse harderian gland has no exact counterpart in man. However, the adenocarcinomas of the harderian gland appear similar to adenocarcinomas of the lacrimal gland that are described clinically.

The iris tumors have not been definitely classified. However, they resemble the appearance of diffusely infiltrating clinical melanomas.

The tumors resembling malignant melanoma of the choroid are markedly anaplastic and undifferentiated. This morphology together with their rapid growth pattern to invade the surrounding structures attests to their local malignancy. No peripheral metastases were detected from the tumors. However, the animals were killed when proptosis was pronounced in an attempt to salvage the globe. In Animal 74-2 fine pigment granules appeared to be liberated by tumor cells in several areas remote from the immediate uveal tract area. The possibility that these tumors originated from the retina or its pigment epithelium after their undergoing metaplasia cannot be ruled out, but this appears unlikely. In future studies dopa reaction tests on fresh tissue are planned in order to evaluate the pigment potential of these cells. Photomicrographs of two human malignant melanomas (figs. 19 and 21) that show sparse pigmentation are presented for comparison with the animal tumors.

The spindle-cell sarcomas of the orbit in mice appear primarily fibrosarcomas and are comparable to primary orbital sarcomas that have been observed clinically.

Summary and Conclusions

Methylcholanthrene, a carcinogenic hydrocarbon, has been introduced into the eyes of several strains of mice by various techniques in a pilot study.

The following tumors were produced: (1) squamous-cell carcinoma of the cornea, (2) squamous-cell carcinomas of the conjunctiva, (3) squamous-cell carcinomas of epithelial downgrowth in the anterior chamber, (4) adenocarcinomas of the harderian gland, (5) diffuse tumors of the iris as yet

unclassified but resembling melanoma, (6) malignant tumors resembling melanomas of the choroid classified epithelioid type, spindle A and spindle B types, and (7) spindle-cell sarcoma of the orbit.

Repeated transplanation of one of these tumors to other mice of the same strain was successful. The local malignancy of the tumors from which transplants were unsuccessful was determined on the basis of their marked local invasiveness to the point of filling and rupturing the globe and invading the orbit and surrounding structures.

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ACKNOWLEDGMENTS

We are indebted to Dr. Frank Figge of the University of Maryland School of Medicine for supplying laboratory animals and facilities during the summer of 1955 to initiate these studies. Dr. Robert J. Dickson, Department of Radiology, The Johns Hopkins Hospital, supervised the radiation experiments and gave advice and many helpful suggestions in these studies. Mr. Belbert Parker of the Wilmer Institute prepared the photomicrographs.

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TEST COMPARING A NEW WITH A CONVENTIONAL ASTIGMATIC CHART*

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Most astigmatic test charts commonly in use are composed of black lines on a white background. These lines may be seen in various patterns such as the letter T, V, an arrow, or a radially arranged "sunburst" dial (fig. 1-A). To determine the presence of astigmatism, the different lines are compared as to their relative blurring or clarity, the criterion being more blur or less blur.

Recently a new test chart[1] was designed using a different pattern involving another criterion for recognition. Essentially, this chart, which was designed by one of the authors (Eastman), is a sunburst dial of black lines with white borders on what appears as a gray background (fig. 1-B). The "gray" background is composed of narrow black and white stripes of equal width. At a distance of 20 feet, these stripes are too narrow to be resolved individually, and the effect is the appearance of a uniform gray field. The test lines themselves have a white border on each side, one half as wide as the black test line. Instead of appearing blurred below the threshold of acuity, the black test line merges imperceptibly into the background. The criterion used with the Eastman chart is the presence, or absence, of the test lines. The test pattern and the background were designed to have equal areas of black and white resulting in equal average reflectance. The reason for using fine stripes for creating a uniformly gray background equal in reflectance to the wide stripe and white border was mainly the technical feasibility of printing a field of *uniform* over-all reflectance.[4,5]

The Eastman chart is based on ideas of Friedenwald[6] and Goldmann.[2] Friedenwald suggested that an astigmatic test chart could be improved by having the test lines bordered with a white hem on a neutral background. Friedenwald tested astigmatic charts similar to the new one described here by photographing them through astigmatic lenses. Raubitschek[7] considered the "white border on a gray background" essential to his arrow chart. Goldmann's idea, developed for the objective determination of visual acuity, led us to use fine stripes for "background" and wide stripes for "signal," both having the same reflectance.

As it was a priori impossible to decide whether or not the Eastman astigmatic chart would be superior to the conventional one, a brief study was made for comparing the sensitivity of the two charts.

METHOD

The charts shown in Figure 1, a conventional "sunburst" dial (A), and the Eastman chart (B) were used in this test. The black test lines on chart A subtended one minute of arc at six m. distance. This is the width of the Snellen 20/20 line on a standard visual acuity chart. The wide black test lines on chart B subtended also one minute of arc, or the equivalent of a Snellen 20/20 line. In addition, another Eastman type chart with lines subtending 45 seconds of arc, approximating a Snellen 20/15 line, was used in this study. For convenience, the charts will be referred to as the conventional chart, East-

* From the Department of Physiology, Basic Health Science Division, Emory University, Atlanta, and the Radiant Energy Effects Laboratory, General Electric Company, Nela Park, Cleveland, Ohio. The experiments were carried out in the Department of Physiology, College of Medicine, Ohio State University, Columbus, Ohio. Address reprint requests to G. A. Brecher, Emory University. Presented before the South-Eastern Section, Winston-Salem, North Carolina, December 6, 1958.

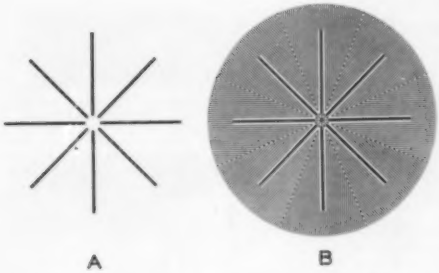

Fig. 1 (Brecher, Lewis, and Eastman). Two astigmatic test charts. (A) Conventional "sunburst" dial. (B) Eastman chart.

man 20/20, and Eastman 20/15 chart, irrespective of the magnification of the testing lens system, which is the same for both types.

The charts were compared by testing the threshold conditions of perception, using a continuously variable-power lens system. This was preferred to stepwise changes which could be produced by using a series of lenses in a trial frame or phoropter. Cylindrical lenses were used instead of spherical lenses, thereby making it possible to vary the power in one meridian while holding it constant in the opposite meridian.

The experimental arrangement is illustrated in Figure 2. A small optical bench was constructed with a movable plus cylindrical lens and a stationary minus cylindrical lens. The lenses were set in simple mounts which could be rotated about the optical axis in measurable degrees. The minus lens was mounted on the end of the bench so that the observer's eye was 15 mm. behind the minus lens. The plus lens was free to be moved on a rider from its primary position in contact with the minus lens to any distance in front of the minus lens. When the two lenses were in contact, the dioptric power of the system was the algebraic sum of the powers of the lenses. As the plus lens was moved away from the minus lens, the dioptric power of the combination increased toward the plus value. The reason is that the effectivity of a plus lens increases as it is moved away from the eye.

Two lenses were chosen so that the dioptric power of the combination could be varied from plus to minus. A +5.0D. cylinder lens was used in this experiment in combination with a −7.0D. cylinder. The lateral separation of the lenses was measured in millimeters, and the total power of the system computed from these data.*

The Eastman charts were tested in the following manner. A two percent homatropine solution was instilled in the subject's eyes in order to rule out effects of accommodative changes. The subject was seated behind the optical bench. A plus spherical lens was positioned in the optical bench at such a distance from a minus spherical lens as to fog the subject slightly. The fog was reduced gradually until one of the lines on the chart became just visible. This line was chosen for further observation, and the axes of the cylinder

* A lens system of this type resembles a Galilean telescope. The test target is slightly magnified depending upon the distance of the convex from the concave lens. Since the degree of magnification was the same for either type of astigmatic chart, it could be neglected in comparison tests.

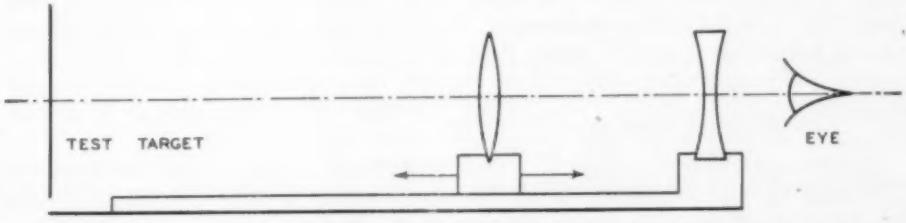

Fig. 2 (Brecher, Lewis, and Eastman). Schematic diagram of experimental arrangement for testing astigmatic charts.

TABLE 1

SUMMARY OF DATA

Subject	20/20 Conventional				20/20 Eastman Chart				20/15 Eastman Chart			
	Dioptric Range from Blur to Clear		Dioptric Range from Clear to Blur		Dioptric Range from Blur to Clear		Dioptric Range from Clear to Blur		Dioptric Range from Blur to Clear		Dioptric Range from Clear to Blur	
1	−0.75	+1.00	−1.65	+1.30	−0.75	+0.75	−1.00	+1.25	+0.06	+0.25	−0.76	+1.12
2	−0.64	+0.25	−1.18	+0.27	−0.63	−0.25	−1.08	+0.25	−0.49	−0.44	−0.96	+0.06
3	+0.81	−1.64	−0.66	+2.93	+0.67	+1.69	+0.25	+2.34	+0.90	+1.24	+0.53	+1.74
4	+0.09	+1.18	−0.90	+2.02	+0.29	+1.13	−0.38	+2.02	+0.81	+0.74	−0.16	+1.38
5	−0.69	+0.31	−1.20	+0.30	−0.67	−0.20	−1.10	+0.30	−0.50	−0.42	−0.99	+0.10
6	−0.10	+1.00	−1.12	+1.63	−0.08	+0.85	−0.85	+1.50	+0.30	+0.45	−0.32	+1.05
7	−0.10	+0.80	−0.65	+1.62	−0.05	+0.77	−0.59	+1.61	+0.47	+0.50	−0.31	+1.10
8	−2.25	−1.38	−2.60	−1.04	−2.45	−1.56	−2.60	−1.38	−2.29	−1.38	−2.57	−1.04
9	−1.67	−0.24	−1.73	+0.09	−0.75	−0.33	−1.29	+0.14	−1.13	−0.93	−1.54	−0.56
10	−1.13	−0.24	−1.54	+0.52	−1.54	+0.52	−1.74	+1.07	−0.33	+0.35	−0.75	+0.57
Average	−0.64	+0.43	−1.32	+0.96	−0.60	+0.34	−1.04	+0.91	−0.22	+0.04	−0.78	+0.55

lenses were placed parallel to the test line without altering the distance between them.

A set of readings was taken by first blurring the test line by fogging the subject with plus power with the lens combination until the line could no longer be distinguished, and then gradually reducing the blur until the line just became visible. The effective power of the lens combination, with the line just visible, was recorded. Then the plus power of the combination was increased just enough to render the line indistinguishable from the gray background, and the effective power recorded. Ten sets of readings were taken.

Similar sets of readings were recorded as the line "just appeared" or "just disappeared" when the effective minus power of the lens system was varied. In this manner the dioptric range was determined in which the test line was visible to the observer. All lens adjustments were made by the subject.

Next, the conventional astigmatic chart (fig. 1-A) was studied under the same conditions. The subject reported when the line was clear or perceptibly blurred rather than visible or invisible. When the limits of the range were exceeded the line appeared only blurred, but was obviously still visible. Each reading was repeated 10 times.

Results and Discussion

The data are presented in Table 1.

A comparison of the figures obtained in the threshold determinations for the conventional chart (fig. 1-A) and the Eastman chart (fig. 1-B) with 20/20 test lines shows that the ranges of clarity for the two charts are approximately equal.

There was, however, a significant difference in the subjective appearance of the threshold. When the range was exceeded—either by too much plus or too much minus effective lens power—the line of the conventional chart appeared only slightly blurred whereas the line of the Eastman chart became completely indistinguishable from the gray background. All subjects agreed that the difference between presence or absence of the line on the Eastman chart was a more precise criterion because of a well defined endpoint.

In order to explore further the sensitivity of the two charts, smaller versions of them, closer to the threshold of visual acuity, were tested. The data recorded for the Eastman chart with 20/15 lines (last group of columns in table 1) showed a narrower range of visibility for the test lines than with the 20/20 lines. The 20/15 modification was much more sensitive than either of the 20/20 charts. A corresponding conventional chart with 20/15 lines could not be tested satisfactorily because none of the subjects could discern reliably differences of "clear" and "blur." The average data comparing the sensitivity of the test charts are graphically represented in Figure 3.

Although the 20/20 Eastman chart was not significantly superior to the conventional chart, the 20/15 Eastman chart proved to be a critical test for evaluating acuity. The use of a testing method with a well defined end-

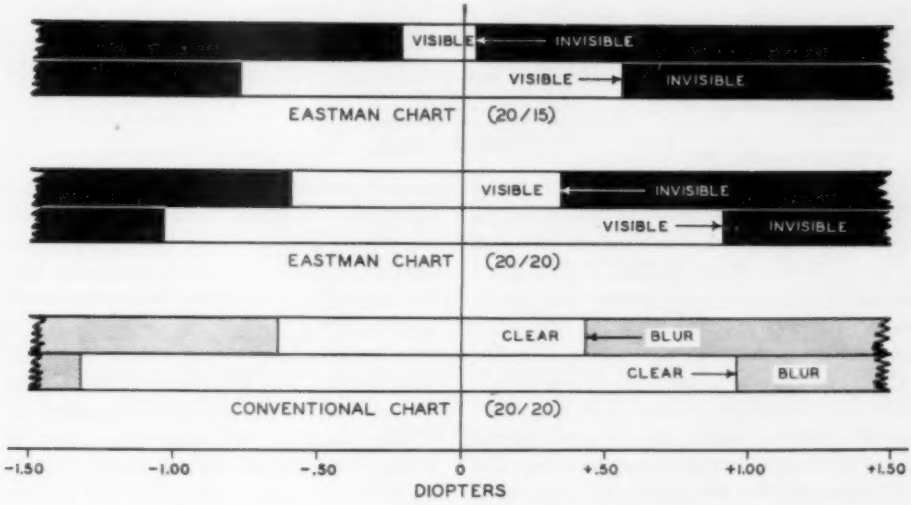

Fig. 3 (Brecher, Lewis, and Eastman). Threshold ranges in diopters obtained with Eastman charts (20/15) and (20/20) and conventional chart (20/20).

point, such as demonstrated with the Eastman astigmatic chart, offers possibilities for other areas of visual acuity testing.

Summary

The new astigmatic chart (Eastman chart) used in this test was a "sunburst" dial of black lines with white borders on a gray background which was composed of narrow black and white stripes of equal width. Beyond a critical distance, these stripes were too narrow to be resolved individually and appeared as a uniform gray field. Instead of appearing blurred below threshold of acuity, the black testline in the axis of the astigmatism merged imperceptibly into the background. The criterion used with the Eastman chart was the presence or absence of the testline. The conventional sunburst dial chart consisted of lines equal in width of the testlines of the Eastman chart. In the conventional chart, the criterion for the presence of astigmatism was "more blur" or "less blur." The charts were compared by testing the threshold conditions of perception using a continuously variable-power lens system. In 10 subjects the dioptric ranges were determined in which the Eastman chart testline was visible to the observer and the conventional chart testline was clear to the observer. It was found that the dioptric ranges were consistently narrower with the Eastman chart. The difference between presence or absence of the line on the Eastman chart was a more percise criterion because of a well defined end-point when compared with the criterion of "more blur" or "less blur" of the line on the conventional chart.

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COMPLETION OF PROJECT 650*

(Special print for subnormal vision)

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The cost of making new fonts of lead type for experimental purposes is prohibitive so a new device was adopted which, although slow in terms of production, enjoys great versatility. It is a special photographic printing device which will print words one letter at a time on strips of photo paper. In this way a book can be printed one line at a time. The lines are then arranged into pages and finally rephotographed onto a metal platten which, when mounted round a drum, will print exactly like the mastic cylinders used on newspaper machines.

The advantage of such a device is that not only can the actual size of the print be controlled, but so can the interletter spacing and the spacing between the lines. The illustration shown here is a composite of a conventional print used for subnormal vision books, and four final choices of what is available in print nearest to the ideal desired without special manufacture. All these were printed in the manner described. The resulting pages were comparable with those seen in any ordinary book.

The specifications of the prints shown were:

No. 1 and No. 4 width to height ratio

4:4.73, limb thickness $\frac{0.024}{0.123} = \frac{1}{5}$

No. 2 and No. 3 width to height ratio

4:5.078, limb thickness $\frac{0.020}{0.137} = \frac{1}{6.5}$

The first two conform most nearly to the ideal 5:5 criteria desired, and they also possess the ideal limb thickness.

The dimensions were translated into minutes of arc, and then the spaces designed accordingly. In prints 1 and 2 they were one-minute arc, and in 3 and 4 they were two-minute arc. No. 4 therefore appears to conform most nearly with the ideal criteria laid down in my previous papers, the letter "O" to be the same height and width, or the width to be not less than 80 percent of height, and limb thickness and spacing between limbs to be 20 percent of height or 25 percent of width, if the height and the width are not identical.

While laboratory experiments were proceeding to determine data on these five illustrated types and spaces, the conventional type, and No. 1 and No. 4, were reproduced with an article on the front page of the *Columbus Dispatch*, readers being asked to carry out a simple testing routine at home, and send their results to me. The response to this was quite voluminous and demonstrated a keen public consciousness of the need for improved type. Some interesting statistics came to light.

Firstly, very fast readers apparently prefer crowded conventionally spaced print. Any type which is spread out to improve legibility reduces their speed considerably. But this connotes a high standard of vision and accommodation, too, and is not applicable to the average older citizens. Of the respondents to this newspaper test 76 percent wore glasses and had worn them for an average of 27 years. The average age of the spectacle wearers was 48 years, and many of them had low acuity and/or a pathologic alteration. Type 4 was unanimously read at the greatest distance and Type 3 occupied second place. However, the nonpathologic and reasonably normal subjects chose conventional spacing for rapid reading. Unfortunately the newspaper had printed this

* From the Department of Ophthalmology, Ohio State University Medical School. Read before the East-Central Section, January 5, 1959, Cincinnati, Ohio.

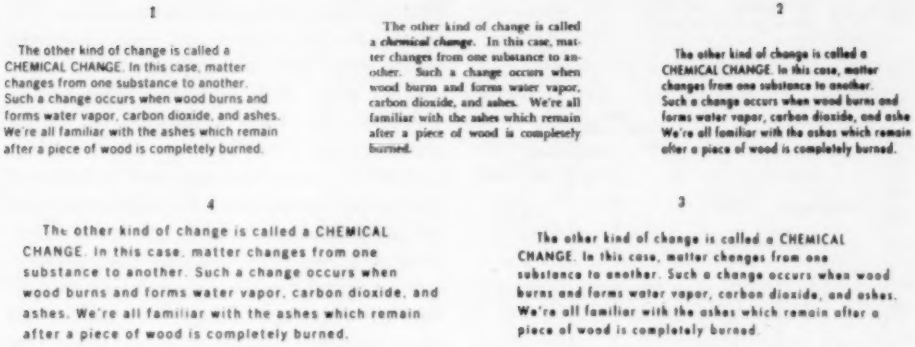
1
The other kind of change is called a CHEMICAL CHANGE. In this case, matter changes from one substance to another. Such a change occurs when wood burns and forms water vapor, carbon dioxide, and ashes. We're all familiar with the ashes which remain after a piece of wood is completely burned.
The other kind of change is called a chemical change. In this case, matter changes from one substance to another. Such a change occurs when wood burns and forms water vapor, carbon dioxide, and ashes. We're all familiar with the ashes which remain after a piece of wood is completely burned.
2
The other kind of change is called a CHEMICAL CHANGE. In this case, matter changes from one substance to another. Such a change occurs when wood burns and forms water vapor, carbon dioxide, and ashes. We're all familiar with the ashes which remain after a piece of wood is completely burned.
4
The other kind of change is called a CHEMICAL CHANGE. In this case, matter changes from one substance to another. Such a change occurs when wood burns and forms water vapor, carbon dioxide, and ashes. We're all familiar with the ashes which remain after a piece of wood is completely burned.
3
The other kind of change is called a CHEMICAL CHANGE. In this case, matter changes from one substance to another. Such a change occurs when wood burns and forms water vapor, carbon dioxide, and ashes. We're all familiar with the ashes which remain after a piece of wood is completely burned.

Fig. 1 (Prince). The five prints used in the experiment described. The top center one is the conventional print. No. 1 and No. 2 have interletter spacing of one-minute arc, and No. 3 and No. 4 have interletter spacing of two-minute arc.

10-percent larger than No. 3, for instance, because it occupied less room.

In the laboratory where both subjects and experiment were under common control, the findings gave much more of the kind of information I was seeking.

1. In the legibility test, 88.6 percent of subjects were able to make the highest scores with print No. 4.

2. With print No. 1, 8.6 percent made the second highest score.

3. With print No. 3, 60 percent made the second highest score.

4. Although 42.9 percent made the second highest score with No. 1, some of these tied for this score with No. 3.

5. With No. 4, 5.7 percent made the second highest score.

6. With print No. 3, 2.3 percent made their highest score.

Briefly then, print No. 4 was the most legible or second most legible print for 94 percent of the subjects. No. 3 was obviously second choice, but No. 1 runs it fairly closely. No. 1 has narrower spacing than No. 3 but it has the superior type face of No. 4. The highest scores therefore go to the superior type face plus the wider spacing. There was a very wide range of visual acuities encountered in the subjects but, among those with low visual acuity, there was no exception to the choice of No. 4 as giving the best results of all the prints shown.

It is difficult to relate this special type to anything a printer understands because his measurements do not refer to the actual sizes of letters but to the dimension of the rectangular lead stems or bases of the letters. There are 72 points to an inch, and 12-point type means that ther are $\frac{72}{12}$ = six lines of print to the inch. Even if the spacing between the lines is increased so that there are only five actual lines of print to an inch it is still 12-point type. The major difficulty is that two kinds of type can both have the same "points" of value and yet have different letter sizes, their lead spaces being identical, however.

We cannot say, therefore, that minutes of arc used in print measurement have an actual relation to printers "points." So the best we can do for the conventional printer is to specify a brand of type and its points value such as "Spartan medium" 18 point, with certain spacing between the letters and another spacing between the lines. But this will have to be worked out carefully for each typeface it is possible to use.

Some investigation was also made on any relationship that might exist between the quality of the paper (especially its reflectance power) and the new type and spacing that might be different from that found in work already accomplished with standard print.

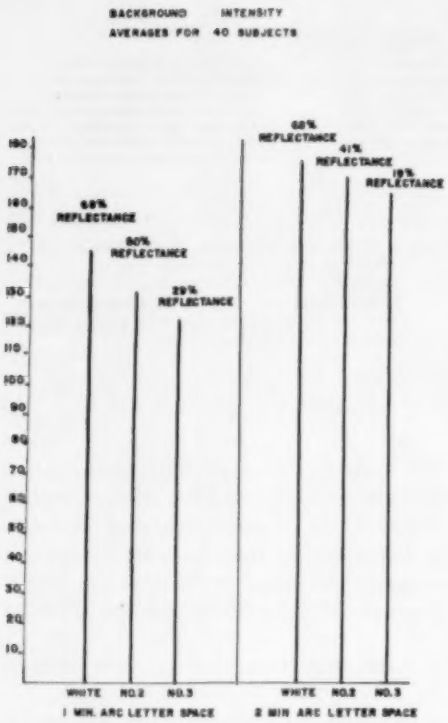

Fig. 2 (Prince). In this graph the ordinate shows the scores achieved by subjects presented with prints having different interletter spacing and different background reflectance of paper.

The results of this were most revealing. The accompanying graph shows that with two-minute arc letter spacing the very least contrasting paper gave better legibility results than even the highest quality contrasting paper did with one-minute arc letter spacing. In fact the wider spacing made background contrast appear to be far less significant than we have hitherto found, all three backgrounds giving closely similar results. However, with the narrower spaced every one of the various background qualities produced a lower legibility standard, and also there was a greater relative legibility disparity between them.

This seems to concur with an earlier finding* that the most important factor in letter legibility is the amount of black in relation to white in a given area. If the quality of paper is reduced in importance when books are being specially printed for the semi-blind it may be possible eventually to print their special books almost as rapidly and cheaply as the standard subnormal vision books have been in the past.

Department of Ophthalmology (10).

* Improvements in letter styles for sight-testing charts. Texas Rep. Biol. & Med., **12**: No. 2 (Summer) 1954.

A FIELD STUDY OF HARDY-RAND-RITTLER COLOR VISION PLATE TEST*

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INTRODUCTION

The placement of, or rejection of, color defective individuals is a matter of considerable concern to the Royal Canadian Air Force, as it is to any large industry.[1]

At the present time the RCAF is using the American Optical Pseudo-Isochromatic plate test as a screening device and the RCAF lantern as a salvaging device. The resultant classifications are, of course, arbitrary and their validity in relation to job placement is meaningful only in the light of experience. Also these classifications in no way reflect the major types of color vision defects, such as protanopia, deutanopia, or tritanopia.

The publishing of the Hardy-Rand-Rittler plate test offered an alternative method of testing.[2] This plate test gives an assessment of the severity and type of color defect,

* From the Institute of Aviation Medicine. Read before the East-Central Section, January 5, 1959, Cincinnati, Ohio.

which could be an aid in the placement of personnel. This report concerns the field trial phase of an assessment of the Hardy-Rand-Rittler plates.

Purpose

From the data of the field trial, an attempt is made to determine what correlation, if any, there is between the H-R-R results and the A-O plate test plus lantern results.[3] The field trial also demonstrates the relative adaptability of the two tests to large scale field testing and indicates the adequacy of the scoring method used, having regard for accuracy and ease of scoring.

Method and Apparatus

Twenty-one RCAF stations participated in the study. Of this total, 14 are Recruiting Units, four are Auxiliary Medical Units, two are representative flying stations, while the remaining one is the Central Medical Establishment. Color vision testing of applicants to the RCAF is routinely carried out by these units. As the tests were performed on applicants to the RCAF, the results obtained cannot be considered indicative of the general population.

Each unit was supplied with the following equipment:

1. 15-plate edition of American optical pseudo-isochromatic plate test.
2. Hardy-Rand-Rittler plate test.
3. RCAF color perception lantern.
4. Macbeth easel lamp.
5. Data forms (app. 1 and 2).

Because one of the aims of the experiment was to evaluate the relative adaptability of the two test procedures, participating stations were supplied with concise written instructions on the procedure to be followed. However, no attempt was made to give individual instruction or to personally supervise the examiners.

Units were instructed that both plate tests were to be illuminated by the Macbeth lamp. The lamp should be placed on a table so that the applicant's line-of-sight was at right angles to the plates when they were set on the lamp. The distance should be approximately 30 inches (just beyond arm's length). The applicant should not be facing an open window or other strong light. Nearby incandescent or fluorescent lights were to be shielded so that they did not illuminate the plates. Window shades in the immediate vicinity were to be drawn, and the cards should be handled with care.

When testing with the A-O plates, the suggested procedure for the examiner to follow was: "ask the subject to please read the numbers." The demonstration plate "A" (a red "12" on a blue background) is then to be shown. The examiner should make all plate changes and the applicant is not to be allowed to trace the patterns or to touch the test plates. He is to be given a reasonable time (2 seconds) for response to each plate. If there is hesitation, he should be asked again to "read the numbers" and, if he fails to respond, the examiner is to present the next plate without comment. With the exception of the demonstration plate, which is always presented first, the plates are to be given in random order. Ten or more correct responses to the 14 test plates constitute a pass and the applicant is to be considered "color vision normal." If five or more incorrect responses are given, including failures to make responses, the subject is to be considered "color vision defective." The demonstration plate is not to be considered in the scoring. In plates on which there are two digits, an incorrect response to either digit constitutes a failure on that plate. All errors are to be indicated by recording the actual response in the square corresponding to the plate number. Correct responses are to be recorded by a check mark in the appropriate square.

If a candidate failed to pass the A-O plate test, instructions were given that he be tested by means of the RCAF color perception lanterns. This lantern employs filters of colored glass and neutral density gelatin. The red filters are Corning No. 2403 heat resistant glass with nonpolished surfaces and are 6.0 mm. $\pm$ 0.5 mm. thick. The green

filters are Corning (4-74) No. 4445 heat resistant glass, with polished surfaces and are 3.0 mm. ± 0.1 mm. thick. The neutral density filters are Wratten neutral density filter No. 96 of densities, 30, 70, 80, and 90. The lamp used is a Mazda C inside frosted, 115-volt 60-watt bulb. The test distance is 20 feet.

Testing consists of 11 presentations of a single light and 11 of two lights. Candidates who make more than one error are classified "unsafe." If only one error is made, they are retested. Providing no errors are made on the second test they are classified "safe." If any error is made on the second test, they are classified "unsafe." In summary, then, the present RCAF classifications are: (a) normal—pass plates; (b) safe—fail plates, pass lantern; (c) unsafe—fail plates, fail lantern.

The instructions given regarding testing by means of the H-R-R plate test were:

Regardless of the results on the A-O plate test, testing by means of the H-R-R plates is to be done under identical test conditions. There are four demonstration plates and 20 test plates. With the subject seated approximately 30 inches from the Macbeth lamp, the four demonstration plates A, B, C, and D are presented. It is to be pointed out that there may be two, one or no colored symbols (triangle, circle, cross), on the plate, and that a symbol may appear in any of the four corners of the plate. It should be explained that the test plates to be presented may have similar designs but different intensities and that all such symbols recognized are to be reported in the following manner; the subject will first report the number of symbols seen, and then identify them (triangle, circle, cross), giving their location as to top left, top right, bottom left, or bottom right. A good way to elicit this information would be to ask "How many symbols do you see?" and then "What and where are they?" A circle is to be represented by O, a triangle by a △ and a cross by X. The location as reported is to be indicated in the numbered square corresponding to the plate number. The test plates are numbered from one to 20 consecutively. The squares corresponding to the demonstration plates A, B, C, and D have been scored to illustrate the method to be used in recording the responses and to indicate the correct responses to these demonstration plates.

Data forms were supplied for recording the responses made by the subject (app. 1 and 2). All data forms were returned to the Institute of Aviation Medicine for interpretation and statistical analysis.

Results

A total of 3,508 applicants to the RCAF were tested by the 21 participating units. The number of returns submitted by each unit is shown in Table 1.

Due to errors and omissions, classification based on the returns from the field presented certain problems. Fifty-one returns indicated either lantern testing was not performed or it was not reported. In addition, 27 subjects were improperly classified by the field on the basis of the recorded responses. For these reasons, a revised or more probable classification is shown in Table 2. These field inaccuracies represent a 2.0 percent error of classification when considering the total group, but a 38 percent error of classification when considering the color defective group. Based on the revised classification, 198 individuals or 5.6 percent of the 3,508 applicants were found to have defective color

TABLE 1

NUMBER OF FIELD RETURNS

Unit	Number of Returns	Unit	Number of Returns
1	187	12	374
2	281	13	159
3	5	14	5
4	365	15	106
5	284	16	67
6	67	17	331
7	1	18	40
8	131	19	270
9	13	20	204
10	517	21	96
11	4		
		TOTAL	3,508

TABLE 2

REVISED CLASSIFICATION

		CVN	CVS	CVU	Unclassified*	Total
Field Classification	CVN	3,293	5	2	2	3,302
	CVS	5	28	2	2	37
	CVU	7	4	83	9	103
	Unclass.	0	6	17	43†	66
		3,305	43	104	56	3,508

* Fifty-one cases made at least five A-O errors but no lantern results were supplied.
† Includes five cases for which H-R-R- results only were received.

vision. There were 323 (9.2 percent) females, none of whom was color-vision defective.

Table 3 illustrates the disposition of the 3,508 applications, of whom 3,186 were accepted and 322 rejected. The rejections were due to all reasons and not necessarily color-defective vision. It should be noted that the rejection of 62 applicants for defective color vision is significantly higher than would have been expected, but it should also be noted that it was possible to accept two thirds of the color-vision defective applicants.

Concerning the information solicited in addition to the actual results of testing, the returns indicated that for two percent of the subjects the Macbeth lamp was definitely not used. In an additional nine percent, no indication was given whether it was used or not. Twelve percent of the subjects wore spectacles while being tested and on 10 percent of the returns no entry was made concerning this. The testing was performed by 143 individuals comprising 57 other ranks, 79 recruiting officers, five physicians, and two civilians. This number of examiners gives a mean of 6.8 examiners per unit. The other ranks comprised 40 percent of the examiners and conducted 41 percent of the tests.

Table 4 indicates the distribution of cases by the H-R-R classification. The left-hand columns show the classifications based on the actual recorded responses. The right-hand columns present a revised classification based on a more probable interpretation.

The more probable interpretation was arrived at by ignoring single or double errors recorded for plates 7 to 20, providing there were no previous wrong responses. Seventy-two such wrong responses were recorded. In addition, it was decided to ignore those color defective classifications which were based solely on a single wrong location of a correct symbol. There were 84 such recordings.

It will be observed that normals comprised 83.5 percent of the total population by this more probable distribution. Thus 16.5 percent of the total population was found to be color-vision defective to some degree, according to the H-R-R test. This would seem high as compared to the 5.6 percent of the same population who were found defective by means of the A-O test. This occurs because four errors are allowed on the A-O test. Table 5 compares the two plate tests. It shows the number of individuals who made one or more errors on either of the tests. It will be noted that on the 14 A-O test plates almost twice as many individuals made errors as on the 20 H-R-R test plates.

Finally, Table 6 shows the detailed relation between testing with the A-O plates in conjunction with the RCAF lantern and the H-R-R test. It must be emphasized that the frequencies upon which the percentages are based are the more probable with respect to both tests. It would seem that the H-R-R

TABLE 3

DISPOSAL OF APPLICATIONS

Color Vision	Accepted	Rejected	Total
Deficient	136	62	198
Normal	3,046	259	3,305
Unknown	4	1	5
	3,186	322	3,508

TABLE 4

DISTRIBUTION OF CASES BY THE H-R-R CLASSIFICATION

H-R-R Classification	Field Returns		More Probable Distribution	
	Number	Percent	Number	Percent
Normal	2,772	79.0	2,928	83.5
Unclassified	424	12.1	411	11.7
Deutan	112	3.2	67	1.9
Protan	81	2.3	49	1.4
Tritan	28	0.8	5	0.1
Tetartan	34	1.0	7	0.2
Mixed	57	1.6	41	1.2
	3,508	100.0	3,508	100.0

TABLE 5

INDIVIDUALS WHO MADE ERRORS

Errors	A-O	H-R-R
Nil	2,142	2,772
1 or more	1,366	736
	3.508	3.508

"normals" and "mild unclassifiables" agree with the RCAF normal classification. Further, it would seem likely that the RCAF "color-vision unsafe" classification is related to degree within an H-R-R group. The mild deutans and mild protans may be associated with "color-vision safe." Very likely some gross error accounts for the three tritans who appear in the "normal" column. For example, it is suspected that the tests for two brothers were confused.

DISCUSSION

It is apparent from the returns that regardless of furnishing explicit instructions, there existed a great diversity of method and procedure. However, it is speculated that these variable test conditions might be representative of color vision testing in general. If this is so, it can be concluded that, regardless of the test equipment used, unless recommended procedures are followed color vision test results will be unreliable. Too often color vision testing is regarded as of secondary importance and therefore is done perfunctorily.

TABLE 6

PERCENTAGE DISTRIBUTION OF SUBJECTS CLASSIFIED NORMAL, SAFE OR UNSAFE BY A-O TEST, AND LANTERN VERSUS H-R-R CATEGORIZATION*

H-R-R Classification	A.O. Test and RCAF Lantern			Total %	Total Cases†
	Normal	Safe	Unsafe		
Normal	99.7	0.3		100.0	2,911
Mild unclassified	91.5	5.5	3.0	100.0	403
Mild deutan	14.3	19.1	66.6	100.0	21
Medium deutan	11.5	3.8	84.6	99.9	26
Strong deutan		11.1	88.9	100.0	9
Mild protan	10.0	20.0	70.0	100.0	10
Medium protan	20.0	5.0	75.0	100.0	20
Strong protan			100.0	100.0	7
Medium tritan	100.0			100.0	2
Strong tritan	100.0			100.0	1
Medium tetartan	40.0		60.0	100.0	5
Strong tetartan			100.0	100.0	1
Mild mixed	68.1	4.5	27.3	99.9	22
Medium mixed		20.0	80.0	100.0	10
Strong mixed	25.0		75.0	100.0	4

* Excluding 56 cases undifferentiated due to incomplete testing.
† Using the "more probable" distribution summarized in Table 4.

Regarding the administration of the H-R-R test, with the exception of recording and interpretation of the results there is no evidence that the test presents any peculiar difficulties. There is, however, little doubt that a pro-forma similar to that used in this experiment is superior to that supplied by the publishers. Even with the pro-forma, there is every likelihood that many of the apparent errors concerning the H-R-R testing are errors of recording. This may be reduced by having the subject do his own recording on the pro-forma.

Conclusions

Reliable color vision testing must be performed by competent examiners under standard conditions.

Only by controlled experiments can the relationship between various color vision tests be established, that is, the A-O test and RCAF lantern versus the H-R-R test.

Provided a simplified score form is used and subjects record their own responses, the Hardy-Rand-Rittler test should present no special administrative problem.

Medical Arts Building.

References

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2. Hardy, L. H., Rand, G., and Rittler, M. C.: H-R-R polychromatic plates. J. Optical Soc. Am., **44**: 509-523 (July) 1954.

3. Schmidt, I.: Comparative evaluation of the Hardy-Rand-Rittler polychromatic plates for testing color vision. USAF School of Aviation Medicine, Project No. 21-31-013.

APPENDIX I

Name_____ Unit_____

Time of day_____ hours. Date_____

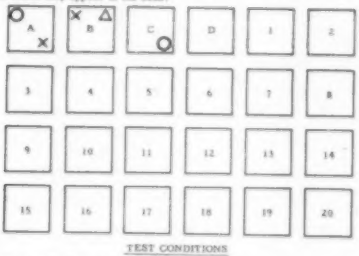
Are glasses habitually worn?_____ Were they worn during test?_____

PSEUDO-ISOCHROMATIC PLATES

Whether correct or incorrect, the examiner is to record the subject's responses, in the square corresponding to plate number. Plates are to be presented in random order, except for demonstration plate "A".

H-R-R PLATES

Examiner is to record symbol(s) and their location(s), as stated by the recruit, in the square corresponding to plate number. Plates are to be presented in the order in which they appear in the book.



TEST CONDITIONS

Illumination: Check all applicable:

Fluorescent; Incandescent; Daylight Dull; Daylight Sunny; Macbeth.

Circle Final Category: CVN CDS CDU Examiner_____ Rank_____

Appendix I (McCulloch, Turnour and Smiley).

APPENDIX II

RCAF COLOUR PERCEPTION LANTERN

Name_____ Date_____

Are Glasses habitually worn?_____ Were they worn during test?_____

NOTE: The actual responses are to be recorded. Check marks for correct responses and X for wrong responses or other such methods are not to be used.

FIRST TEST		
Knob Positions	One Aperture	Both Apertures
1	G	GR
2	R	RW
3	W	WG
4	G	GW
5	W	WW
6	W	WR
7	R	RG
8	G	GG
9	G	GW
10	W	WW
11	W	WG

SECOND TEST		
Knob Positions	One Aperture	Both Apertures
1	G	GR
2	R	RW
3	W	WG
4	G	GW
5	W	WW
6	W	WR
7	R	RG
8	G	GG
9	G	GW
10	W	WW
11	W	WG

Circle final category: CVN CDS CDU Examiner_____ Rank_____

Unit_____

Signature of Recruit_____

Appendix II (McCulloch, Turnour and Smiley).

THE INFLUENCE OF TONOGRAPHY OF ONE EYE ON THE TONOGRAPHIC READINGS OF THE FELLOW EYE*

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INTRODUCTION

The phrase "ophthalmotonic consensual reaction" (Weekers[1,2]) refers to some peculiar phenomena which, after stimulation of one eye, appear in the contralateral eye. They are the result of a relationship or interdependence between symmetric organs.

It has long been known that a stimulus applied to one eye may cause a change in the intraocular pressure of the other eye, and that the variety of such stimuli is very large. Magitot,[3] Morax and Girard,[4] Leplat,[5,6] Larsson,[7] and Linnér[8] described a decrease of the intraocular pressure in one eye after compression or contusion of the contralateral eye. Much of this and other work in this field[9] published before 1938 has been reviewed by Ascher.[10]

In 1954 Bárány and Wirth[11] discovered that a paracentesis performed on one eye of rabbits which had been subjected to intravenous perfusion of para-amino hippuric acid, in some instances produced a complete or almost complete cessation of flow of aqueous humor in the opposite eye.

Becker[12] obtained a flat tonogram on one eye when the other eye was strongly compressed. Here too it was suggested that the flatness was the result of cessation of both inflow and outflow.

Kornblueth and Linnér,[13] after performance of tonography over a period of four minutes on one eye of a rabbit, observed on the other eye an intraocular pressure that was lower by nine percent. The rate of aqueous flow was decreased by 20 percent. A similar phenomenon has been observed in human eyes.

From the experiments of Prijot and Stone[14] it appeared that in rabbits, compression of one eye is followed by a decrease in the intraocular pressure and in aqueous flow of the contralateral eye. This phenomenon was not found in atropinized rabbits, a fact which suggested the possible role of the parasympathetic nervous system in this reaction. The authors pointed out the possibility that in human eyes conventional clinical tonography of the first eye might affect the tonographic values of the second eye.

Similar observations were made by Ballintine,[15] who stated that "Frequently the intraocular pressure of the second eye decreases spontaneously while tonography is being performed on the first and occurs whether left or right eye is used first. The cause of the effect is unknown. It can frequently be avoided or reduced by waiting five or more minutes before examining the second eye. Usually the effect is small and does not seriously interfere with interpretation."

Stocker[16] showed that if a second tonometric reading is taken four minutes after the first one, while the electronic tonometer has been resting on the other eye during the four minute interval, the pressure in the eye on which tonography was not performed averaged 2.37 mm. Hg lower for 12 nonglaucomatous eyes, and 3.25 mm. lower for eight glaucomatous eyes. There was a rise of pressure in one normal and one glaucomatous eye after the fellow eye had been subjected to tonometry for four minutes.

* From the Clinica Oculistica, Ospedale Maggiore, and the Department of Ophthalmology, College of Medicine, University of Cincinnati. Aided by United States Public Health Grant B-158. Presented before the East-Central Section, Cincinnati, Ohio, January, 1959.

Our purpose in the following study was to provide answers to the following questions:

To what extent does tonography performed on one eye of a subject affect the tonographic values obtained for the other eye? What are the average and extreme values of the changes? Do they occur in all normal eyes, or only in some? How do the changes in eyes with chronic simple glaucoma compare with those in normal eyes?

We used only two time intervals, two and four minutes, between tonographies, deferring a full answer to the question of how much time must elapse after tonography on the first eye before tonography can be performed on the second eye without being influenced by the first tonography.

Method

Our studies were carried out on 32 normal eyes (16 subjects) and 48 eyes affected by chronic simple glaucoma (24 subjects). The diagnosis of chronic simple glaucoma was established on the basis of increased intraocular pressure, substantiated by a complete ophthalmologic examination, and, when necessary, by provocative tests. Eyes with absolute glaucoma were not included. The subjects with normal eyes had a physical examination prior to testing, and only completely healthy persons were included. The ages of the normal group were comparable to those of the glaucomatous group.

The tonographic studies were conducted using a standardized Mueller electronic tonometer with the recording unit. All equipment was carefully cleaned and calibrated before each determination. Tonography was performed with the subject in the supine position, clothing loosened around the neck, and eyes in the primary position. The lids were retracted by means of a nonmagnetic self-retaining speculum. Great care was taken to avoid massage of the eye during the testing. For the glaucomatous eyes the use of miotics was discontinued for a period of 48 hours prior to and during the test.

On the first day tonography was performed first on the right eye, then on the left eye. After two or three days, using the same subjects, tonography was performed first on the left eye, then on the right eye. For each subject the tonographies were performed at the same hour of the day in order to minimize the diurnal variation effect.

The basic data obtained are presented in Tables 1 to 4.

Two series of experiments were performed. In the first series (six normal subjects, seven patients affected by chronic

TABLE 1

TONOGRAPHIC VALUES FOR NORMAL EYES: WITH A TIME INTERVAL OF TWO MINUTES BETWEEN TONOGRAPHY ON THE FIRST EYE AND TONOGRAPHY ON THE SECOND EYE

Case		O.D. before O.S.				O.S. before O.D.			
		P_t	C	F	P_0	P_t	C	F	P_0
1	O.D.	21.46	0.26	4.75	18.27	16.38	0.26	3.84	14.79
	O.S.	22.60	0.27	5.21	19.30	19.10	0.26	4.29	16.50
2	O.D.	24.30	0.24	4.97	20.71	29.01	0.25	6.22	24.89
	O.S.	21.44	0.23	3.20	18.25	29.50	0.21	4.29	25.21
3	O.D.	25.66	0.29	6.36	21.96	21.80	0.31	5.75	18.57
	O.S.	21.48	0.35	6.49	18.57	25.40	0.41	8.86	21.61
4	O.D.	21.08	0.28	4.19	18.57	17.90	0.30	4.68	15.60
	O.S.	18.82	0.30	4.97	16.26	19.20	0.30	4.93	16.45
5	O.D.	21.85	0.38	7.03	18.50	22.95	0.43	8.86	19.45
	O.S.	16.25	0.21	3.01	14.34	24.10	0.22	4.27	20.61
6	O.D.	19.10	0.33	5.44	16.50	18.90	0.31	4.05	16.30
	O.S.	16.90	0.36	5.33	14.82	21.80	0.39	7.24	18.57

TABLE 2

TONOGRAPHIC VALUES FOR EYES AFFECTED BY CHRONIC SIMPLE GLAUCOMA: WITH A TIME INTERVAL OF TWO MINUTES BETWEEN TONOGRAPHY ON THE FIRST EYE AND TONOGRAPHY ON THE SECOND EYE

Case	O.D. before O.S.				O.S. before O.D.			
	P_t	C	F	P_0	P_t	C	F	P_0
1 O.D.	36.20	0.10	3.11	31.15	33.78	0.11	3.21	29.22
O.S.	31.90	0.12	3.30	27.53	35.20	0.16	4.88	23.50
2 O.D.	32.80	0.08	2.26	28.35	27.70	0.09	2.11	23.50
O.S.	29.90	0.06	1.43	23.88	32.00	0.07	1.91	27.32
3 O.D.	32.80	0.12	3.50	28.35	23.00	0.12	2.54	19.58
O.S.	29.90	0.17	4.08	25.61	29.90	0.17	4.35	25.61
4 O.D.	38.64	0.12	3.95	32.94	37.20	0.07	2.26	32.32
O.S.	34.60	0.16	5.44	29.61	33.50	0.14	4.05	28.95
5 O.D.	26.50	0.23	5.18	22.55	24.60	0.25	5.25	21.01
O.S.	25.70	0.22	4.81	21.92	24.00	0.18	3.67	20.41
6 O.D.	39.16	0.04	1.36	34.02	38.50	0.10	3.35	33.52
O.S.	37.20	0.08	2.58	32.32	41.30	0.18	6.21	34.77
7 O.D.	24.30	0.13	2.69	20.71	24.60	0.13	2.73	21.01
O.S.	22.40	0.12	2.28	19.00	23.80	0.14	2.83	20.28

TABLE 3

TONOGRAPHIC VALUES FOR NORMAL EYES: WITH A TIME INTERVAL OF FOUR MINUTES BETWEEN TONOGRAPHY ON THE FIRST EYE AND TONOGRAPHY ON THE SECOND EYE

Case	O.D. before O.S.				O.S. before O.D.			
	P_t	C	F	P_0	P_t	C	F	P_0
1 O.D.	25.36	0.20	4.32	21.61	26.42	0.17	3.84	22.61
O.S.	23.00	0.18	3.52	19.58	29.20	0.15	3.60	24.04
2 O.D.	25.70	0.26	5.68	21.90	25.00	0.34	7.24	21.31
O.S.	26.50	0.28	6.31	22.55	25.74	0.35	7.70	22.02
3 O.D.	16.90	0.24	3.75	14.82	17.10	0.19	2.81	14.80
O.S.	18.50	0.21	3.36	16.01	13.40	0.18	2.19	12.21
4 O.D.	21.50	0.19	3.46	18.25	22.10	0.24	4.13	17.32
O.S.	23.00	0.23	4.50	19.58	22.60	0.28	5.44	19.45
5 O.D.	23.40	0.24	4.80	20.03	22.12	0.18	3.39	18.87
O.S.	21.80	0.26	4.82	18.57	26.42	0.23	5.20	22.61
6 O.D.	23.30	0.37	7.41	20.03	25.00	0.37	7.88	21.31
O.S.	21.46	0.35	6.39	18.27	26.04	0.35	7.65	22.61
7 O.D.	22.60	0.28	5.44	19.45	20.24	0.34	5.97	17.56
O.S.	23.40	0.38	6.84	17.76	22.76	0.39	7.47	19.43
8 O.D.	18.90	0.21	3.42	16.30	16.25	0.21	3.01	14.34
O.S.	17.90	0.22	3.43	15.60	15.60	0.27	3.73	13.84
9 O.D.	22.95	0.27	5.25	19.45	21.80	0.22	4.07	18.52
O.S.	18.50	0.24	3.84	16.01	23.96	0.25	5.10	20.41
10 O.D.	23.00	0.24	4.69	19.58	25.40	0.26	5.37	20.76
O.S.	20.24	0.23	4.03	17.56	24.20	0.29	5.26	21.61

TABLE 4

TONOGRAPHIC VALUES FOR EYES AFFECTED BY CHRONIC SIMPLE GLAUCOMA: WITH A TIME INTERVAL OF FOUR MINUTES BETWEEN TONOGRAPHY ON THE FIRST EYE AND TONOGRAPHY ON THE SECOND EYE

Case	O.D. before O.S.				O.S. before O.D.			
	P_t	C	F	P_0	P_t	C	F	P_0
1 O.D.	25.66	0.29	6.36	21.96	25.82	0.19	4.18	22.01
O.S.	25.66	0.19	4.17	21.96	29.56	0.13	3.27	25.21
2 O.D	48.05	0.08	2.88	36.09	38.64	0.15	5.54	32.94
O.S.	31.30	0.10	2.69	26.95	33.04	0.20	5.58	27.98
3 O.D.	34.70	0.12	3.60	30.02	32.30	0.17	4.74	27.93
O.S.	31.90	0.12	3.30	27.53	30.03	0.12	3.12	26.01
4 O.D.	23.48	0.14	2.80	20.00	25.24	0.15	3.29	21.95
O.S.	25.93	0.14	3.09	22.09	29.90	0.13	2.10	24.49
5 O.D.	31.90	0.20	5.50	27.53	27.94	0.15	3.61	24.12
O.S.	28.70	0.15	3.69	24.64	28.70	0.10	2.44	24.49
6 O.D.	29.50	0.12	3.02	25.21	32.80	0.08	2.26	28.35
O.S.	33.74	0.15	4.38	29.22	33.50	0.17	4.96	29.22
7 O.D.	23.40	0.21	4.36	20.83	21.48	0.25	4.64	18.57
O.S.	24.90	0.22	4.68	21.31	22.12	0.25	4.75	19.00
8 O.D.	31.30	0.28	7.01	25.09	31.90	0.20	5.50	27.53
O.S.	31.10	0.24	5.96	24.85	34.70	0.09	2.70	30.03
9 O.D.	33.00	0.21	5.79	27.85	33.50	0.25	7.65	30.62
O.S.	31.90	0.14	3.85	27.53	30.30	0.12	3.13	26.11
10 O.D.	32.00	0.25	6.83	27.32	32.00	0.14	3.82	27.32
O.S.	32.74	0.17	4.79	28.20	31.52	0.12	3.21	26.90
11 O.D.	30.30	0.16	4.16	26.01	27.94	0.17	4.06	23.92
O.S.	30.30	0.23	5.98	26.01	28.70	0.15	3.69	24.64
12 O.D.	39.10	0.15	5.10	34.02	34.60	0.18	5.32	29.61
O.S.	38.65	0.20	6.70	33.52	35.16	0.22	6.56	29.82
13 O.D.	30.30	0.17	4.42	26.01	27.22	0.22	5.10	23.20
O.S.	28.75	0.19	4.88	24.64	24.60	0.21	4.73	22.55
14 O.D.	32.80	0.12	3.50	28.35	30.30	0.15	3.90	26.01
O.S.	29.90	0.17	4.08	23.83	34.70	0.19	5.70	30.02
15 O.D.	24.60	0.15	3.38	22.55	31.52	0.30	8.07	26.92
O.S.	24.60	0.13	2.93	22.55	32.00	0.24	6.55	27.32
16 O.D.	30.10	0.20	5.18	25.61	32.30	0.14	3.91	27.93
O.S.	32.30	0.16	5.06	27.93	30.46	6.08	2.10	26.35
17 O.D.	48.05	0.10	3.60	36.09	42.90	0.14	4.40	31.36
O.S.	44.00	0.13	4.42	34.00	43.60	0.15	5.84	32.16

simple glaucoma) the time between the first and second tonography was two minutes. In the second series (10 normal subjects and 17 patients with chronic simple glaucoma) the time between the first and second tonography was four minutes. These figures are summarized in Table 5.

The data were analyzed statistically by means of the F- and t-tests as described by Goulden.[17]

RESULTS AND DISCUSSION

RESULTS FOR TWO-MINUTE INTERVALS

The results of the analyses for the tonographies with two-minute intervals are presented in Tables 6 (normal eyes) and 7

(glaucomatous eyes). These tables show the averages of the tonographic values and whether the differences are statistically significant at the five percent level.

All of the intraocular pressure averages were higher for the eyes on which tonography was performed first. However, none of the differences between the intraocular pressure averages was significant by the t-test. Similarly, none of the differences between the facility averages or the flow averages was significant by the t-test.

TABLE 5

NUMBER OF NORMAL AND GLAUCOMATOUS EYES STUDIED AT TWO TIME INTERVALS

Intervals between Tonographies (Min.)	Number of Pairs of Eyes Studied	
	Normal	Glaucomatous
2	6	7
4	10	17

The F-test, which compares the variances, showed no significant differences for any of the tonographic values.

The maximum changes of tonographic values found with two-minute intervals between tonographies are presented in Table 8. This table also shows the number of eyes in which the tonographic values increased, decreased, or remained unchanged. It is to be noted that the intraocular pressures decreased in the second eye considerably more often than they increased.

RESULTS FOR FOUR-MINUTE INTERVALS

The results of the analyses for the tonographies with four-minute intervals are presented in Tables 9 (normal eyes) and 10 (glaucomatous eyes). These tables show the averages of the tonographic values and whether the differences are statistically significant at the five-percent level.

TABLE 6

AVERAGE TONOGRAPHIC VALUES OBTAINED FOR NORMAL EYES WITH TWO-MINUTE INTERVALS BETWEEN TONOGRAPHIES

	P_0	C	F
Normal right eyes			
Right eye first	19.10	0.297	5.457
	NS	NS	NS
Right eye second	18.27	0.310	5.566
Normal left eyes			
Left eye first	19.82	0.298	5.647
	NS	NS	NS
Left eye second	16.92	0.287	4.702

NS—no statistically significant difference at the five percent level by either the F- or t-test.

TABLE 7

AVERAGE TONOGRAPHIC VALUES OBTAINED FOR GLAUCOMATOUS EYES WITH TWO-MINUTE INTERVALS BETWEEN TONOGRAPHIES

	P_0	C	F
Glaucomatous right eyes			
Right eye first	28.31	0.1171	3.150
	NS	NS	NS
Right eye second	25.73	0.1257	3.064
Glaucomatous left eyes			
Left eye first	25.84	0.1486	3.417
	NS	NS	NS
Left eye second	25.69	0.1329	3.986

NS—no statistically significant difference at the five percent level by either the F- or t-test.

TABLE 8

FREQUENCY AND MAGNITUDE OF CHANGES OF TONOGRAPHIC VALUES WHEN TONOGRAPHY WAS PERFORMED FIRST ON THE OTHER EYE, WITH TWO-MINUTE INTERVALS BETWEEN TONOGRAPHIES

	P_0	Maximum Change	C	Maximum Change	F	Maximum Change
Normal eyes						
No. of increases	3	4.2	6	0.05	5	1.83
No. of decreases	9	7.0	4	0.06	7	2.37
No. with no change	0		2		0	
Glaucomatous eyes						
No. of increases	4	4.0	7	0.06	6	1.99
No. of decreases	9	8.8	5	0.10	8	3.63
No. with no change	1		2		0	

Here again, all of the intraocular pressure averages were higher for the eyes on which tonography was performed first. However, none of the differences between the intraocular pressure averages was significant by the t-test. This same test showed that the average flow obtained on normal left eyes was significantly higher when tonography was performed on these eyes first. The average flow found with normal right eyes was also higher when tonography was performed on these eyes first, but not significantly so. There were no significant differences found between the average flow values for glaucomatous eyes. The averages for the facility values also showed no significant differences by the t-test.

As indicated by the F-test, none of the variance pairs were significantly different except for intraocular pressure of normal left eyes. When tonography was performed on the normal left eyes first the variance of the intraocular pressures was 3.4 times greater than when tonography was performed on the same eyes four minutes after the right eyes.

The maximum changes of tonographic values found with four-minute intervals between tonographies are presented in Table 11. This table also shows the number of eyes in which the tonographic values increased, decreased, or remained constant. In this table again we find that the tonographic values increased in some eyes and decreased in others, so that no hard and fast rules can be given regarding the effect of the order in which tonography is performed.

The only regularities which appear in these data are the decrease in flow in normal left eyes when tonography is performed first on the right eyes, and that the average intraocular pressures are slightly lower for those

TABLE 9

AVERAGE TONOGRAPHIC VALUES OBTAINED FOR NORMAL EYES WITH FOUR-MINUTE INTERVALS BETWEEN TONOGRAPHIES

	P_0	C	F
Normal right eyes			
Right eye first	19.12	0.250	4.822
	NS	NS	NS
Right eye second	18.74	0.252	4.771
Normal left eyes			
Left eye first	19.80	0.274	5.334
	S(F)	NS	S(t)
Left eye second	18.17	0.258	4.704

NS—no statistically significant difference at the five percent level by either the F- or t- test.
S(F)—variances significantly different at the five percent level.
S(t)—means significantly different at the five percent level.

TABLE 10

AVERAGE TONOGRAPHIC VALUES OBTAINED FOR GLAUCOMATOUS EYES WITH FOUR-MINUTE INTERVALS BETWEEN TONOGRAPHIES

	P_0	C	F
Glaucomatous right eyes			
Right eye first	27.09	0.1735	4.558
	NS	NS	NS
Right eye second	26.48	0.1782	4.705
Glaucomatous left eyes			
Left eye first	26.61	0.1571	4.084
	NS	NS	NS
Left eye second	26.27	0.1665	4.391

NS—no statistically significant difference at the five percent level by either the F- or t-test

groups of eyes on which tonography was performed second. Although the differences between the average intraocular pressures are not significant for the small sample sizes used in this work, it is possible that tests on larger samples would show the differences to be significant. In individual cases either increases or decreases of substantial amounts can occur in all of the tonographic values. Consequently a time interval greater than four minutes should elapse between tonographies performed on eyes of the same individual.

Granting that tonography does produce consensual ophthalmotonic reaction in some subjects, it is of interest to summarize the reasons which have been suggested for such effects.

Most authors suggest that such reactions are due to a nervous reflex from one eye to the contralateral eye. Some think that the reflex is direct (Magitot[3] Weekers[1,2]). However, Birch-Hirschfeld[18] has pointed out the possible role of a central nervous center. Prijot and Stone[16] have suggested the possible role of the parasympathetic nervous system.

Consensual changes of the intraocular pressure have usually been ascribed to a reflex vascular reaction, and it is possible that the consensual change noted in the flow is also dependent on a vascular reflex (Bárány and Wirth;[11] Linnér;[8] Leplat[5,6]). The first phenomenon, according to Leplat, can be ascribed to a great increase in permeability of the vascular endothelium and of the ciliary epithelium. One possibility would be that a great increase in permeability takes place in the posterior chamber of the second eye. The excess osmotic pressure of the aqueous would thus be reduced or abolished and with it the probable driving force for flow (Bárány and Wirth[11]).

Whatever the reasons, tonography per-

TABLE 11

FREQUENCY AND MAGNITUDE OF CHANGES OF TONOGRAPHIC VALUES WHEN TONOGRAPHY WAS PERFORMED FIRST ON THE OTHER EYE, WITH FOUR-MINUTE INTERVALS BETWEEN THE TONOGRAPHIES

	P_0	Maximum Change	C	Maximum Change	F	Maximum Change
Normal eyes						
No. of increases	7	3.8	7	0.08	6	1.56
No. of decreases	12	4.4	10	0.07	14	1.41
No. with no change	1		3		0	
Glaucomatous eyes						
No. of increases	16	4.3	19	0.15	21	4.69
No. of decreases	15	6.2	14	0.11	13	3.62
No. with no change	3		1		0	

formed first on normal right eyes definitely decreases the flow in the corresponding normal left eyes, on the average. With larger samples a corresponding reaction on the flow from normal right eyes when tonography is performed first on normal left eyes could probably be demonstrated. More data will also be necessary to show conclusively by our experimental method that intraocular pressures, on the average, are reduced by tonography on the fellow eye.

Conclusions

The data indicate, but with these small samples do not prove, that in a group of individuals the average intraocular pressure for either right or left eyes, normal or glaucomatous, is decreased by prior tonography on the contralateral eyes. The decreases ranged up to 7.0 mm. for normal eyes and 8.8 mm. for glaucomatous eyes, but increases up to 4.2 mm. for normal eyes and 4.3 mm. for glaucomatous eyes were also observed.

The average facilities are not significantly changed in either right or left eyes by prior tonography on the contralateral eyes. This is true for both normal and glaucomatous eyes.

The average flow in normal left eyes is significantly higher (five percent level) when tonography is performed on these eyes first, with four minute intervals. The trend is in the same direction for normal right eyes with the same intervals, but the difference is not significant. None of the differences between average flows is significant for normal eyes at two-minute intervals, or for glaucomatous eyes at either interval.

In individual subjects both increases and decreases are noted for all tonographic values following tonography on the contralateral eye. Therefore no general rule can be given to cover all cases.

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A CONTRIBUTION TO THE INJECTION TECHNIQUE FOR STUDYING RETINAL BLOOD VESSELS*

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Each of the various methods which has been used to study the retinal vascular system has one or more disadvantages. Injection of the vessels with India ink[2, 14] or with other dyes[6–8, 10] either through the arteries or veins displays the shape and caliber of the vessels but leaves invisible the structural details of their walls. In corrosion cast preparations[3] an indigestible material (for example, latex[1, 5, 9, 12] or vinylacetate[11, 13]) is injected into the vessels and, after allowing for a period of hardening, the surrouding tissue is digested away. These preparations also provide a satisfactory means of studying the vascular tree in its continuity, but give no information on the structure of the vessel walls. Histologic sections, on the other hand, using such stains as the PAS (Hotchkiss) or Feyerter's procedure, reveal only short distances along the individual vessels and give little impression of the vascular tree in its continuity.

In our technique we inject the retinal vessels with silver nitrate and then expose the whole retina to a bright light. The portions of the vessel walls which imbibe the silver nitrate, particularly the reticular fibers and the intracellular cement substances, are stained brown. Thus the retinal vascular tree can be studied in its continuity and also some structual details observed in the vessel walls.

INTRA-ARTERIAL INJECTION OF THE RETINAL VASCULAR TREE WITH SILVER NITRATE IN RATS

For injection purposes we use a pressure pump attached to a Wolff bottle. A mercury manometer allows for reading the injection pressure. Albino rats are given 0.1 cc./100 gm. body weight of heparin intravenously. They are then killed with an overdose of ether. The abdominal and thoracic cavities are opened and a glass tube is tied into the descending aorta, the tube pointing toward the heart. The tube is connected to a Wolff bottle containing the solution to be injected. Both the jugular and the inferior caval vein are incised to provide adequate outflow on the venous side. The animal is then perfused with saline at pressure of 110 mm. Hg until clear saline returns through the jugular veins. Next, distilled water is injected at an initial pressure of 110 mm. Hg. The water is left in the vessels for five minutes by clamping the connecting tube from the Wolff bottle. The clamp is then removed and the flow resumed. Any blood not washed out by the saline is hemolized by the water and flushed from the system. The infusion of distilled water is followed by injection of five-percent formalin in saline to fixate the blood vessels. The formalin-saline is in turn washed out with distilled water.

A three-percent silver-nitrate solution is injected at a pressure of 120 mm. Hg until a liberal outflow from the jugular veins is observed. The animals' eyes are then exposed to sunlight or a powerful (500 watt) electric bulb. The exposure time, depending on the light source, is one to three minutes. During the period of exposure to light the corneas are kept moist with drops of saline. The superfluous silver nitrate solution is finally washed out from the vascular system, using water, and the cannula is disconnected from the aorta.

The eyes are enucleated and stored in 10-percent formalin-saline for one to two days. They are sectioned equatorially just behind the limbus, and the retina teased from the choroid. The retinas are transferred to glass slides on which a few drops of 10-percent

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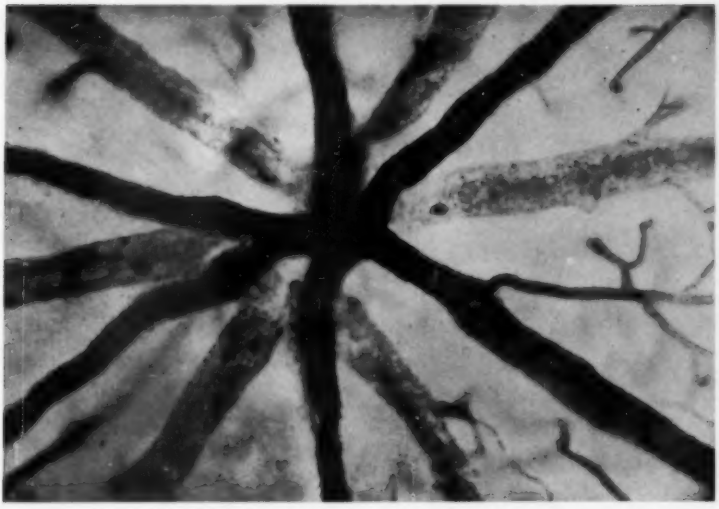

Fig. 1 (Hausler and Sibay). Region of the optic disc, with five main arterioles and veins in distribution peculiar to the rat.

formal-saline have been placed. In order to spread a retina on the slide a few incisions are made meridionally. Permanent slides can be made by subjecting the tissue to the usual histologic clearing procedures. The vessels in these preparations can be viewed from either the vitreal or choroidal side.

Figure 1 shows the region of the optic disc with the five main arterioles and veins in a distribution peculiar to the rat. Arteries show a ribbonlike radial pattern due to the staining of the perimysial fibrils.[4] In capillaries and venules argyrophilic fibers are distributed more irregularly in both an axial and radial pattern.

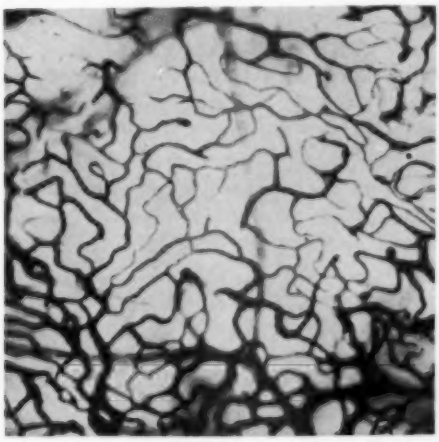

Fig. 2 (Hausler and Sibay). Some of the retinal capillary loops are apparent.

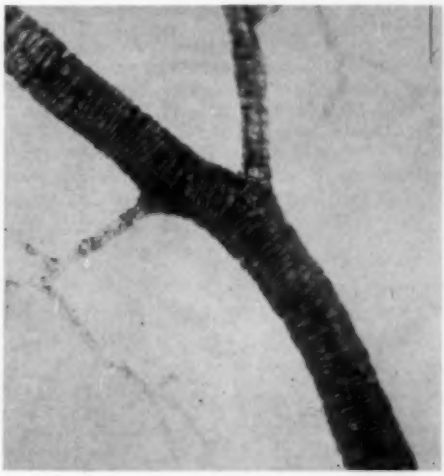

Fig. 3 (Hausler and Sibay). Capillaries branching off from one of the main arterioles.

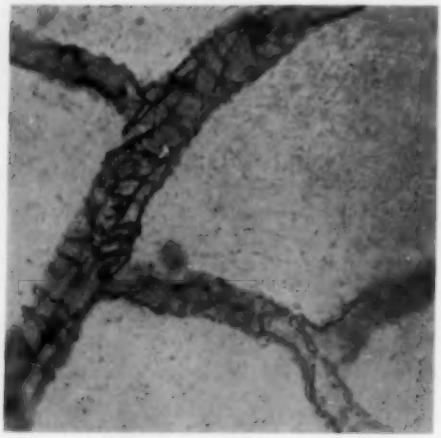

Fig. 4 (Hausler and Sibay). The ending of a capillary into a venule.

In Figure 2 some of the retinal capillary loops are apparent. Two distinct capillary networks were observed in the rat, one superficially in the nerve-fiber layer and one deep at the border of the inner nuclear and outer plexiform layer. The deep network is denser than the superficial one. The figure shows vessels in the superficial layer. The deep network can be dimly seen.

Figure 3 shows one of the main arterioles giving off capillaries. The radial distribution of argyrophilic fibers which is clearly seen in the arteriole changes into an axial pattern on the capillary.

Figure 4 shows the ending of a capillary into a venule. While there is an axial pattern of argyrophilic fibers in the capillary the venule shows both an axial and radial pattern.

Summary

An injection technique is described which allows the study of the retinal vascular tree in its continuity. It is possible to observe the shape and caliber of the vessels and structural details in their walls.

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VERGENCE AND ACCOMMODATION: III. PROPOSED DEFINITIONS OF THE AC/A RATIOS*

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INTRODUCTION

The increase of accommodation of one eye is invariably accompanied by an inward rotation (vergence) movement of the other (occluded) eye. Some of the characteristics of this movement have already been described.[1, 2] The movement seems to be more closely related to the change in accommodation than to the change in its stimulus.[3] In the present paper a way in which this relation can be quantified will be developed. In a series of experiments, the magnitude of the accommodative responses and vergence movements (as measured by the horizontal phoria) were determined as the accommodation stimulus was varied in essentially the same manner as that described in a previous paper.[4] These present experiments differ from them only in that measurements were made over a much wider range of stimuli than those attempted previously.

METHOD

Four prepresbyopic adult males served as subjects. The two arms of the haploscope previously described[4] were adjusted so that the subject saw (with his right eye) a test chart at the other end of the room (3.45 m. from his eyes) through (1) a half silvered mirror, (2) a lens which determined the accommodation stimulus, (3) his lens correction, and (4) a 2.0 mm. in diameter artificial pupil.

By reflection in the half silvered mirror, this eye saw a small point source of light viewed as a stigmatoscope by means of a Badal optometer arrangement. The subject could move the object source along the right arm of the haploscope and find the point conjugate to the retina by setting the light until it was seen sharply focused in the center of the chart of letters. The mirror before the left eye was prevented from transmitting any light at all so that this eye did not view the chart but merely a similar point source of light slightly below that one seen by the right eye. This arm of the haploscope, however, was permitted to rotate freely around the center of rotation of the left eye.

The task of the subject consisted in (1) adjusting this arm of the haploscope so that the two stigmatoscope lights were vertically aligned over the center of the test chart, and in (2) setting the source of light on the right arm of the haploscope so that it was in sharpest focus, while simultaneously (3) actively reading the smallest visible row of letters on the test chart. In this way simultaneous measurements were made of the horizontal heterophoria (by the vergence scale of the left arm of haploscope), of the accommodation stimulus (by the magnitude of the lens placed in the lens cell before the right eye), and of the accommodation response (by the position of the object light source of the stigmatoscope on the right arm of the haploscope). This process was repeated three times in any given experimental session for each accommodation stimulus.

The accommodation stimulus was varied in one-half diopter steps from a negative stimulus of 4.0 diopters to a positive stimulus of 10.5 or 11 diopters. With the usual arrangement of the haploscope higher positive

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accommodation stimulus values were impossible because the associated vergence movements were usually so large that the subject's nose interfered with the inward rotation of the left haploscope arm. To obviate this difficulty a rather strong (32$^{\Delta}$) trial case prism was placed base out in the lens cell of the left arm of the haploscope.*

Suitable rest periods were interspersed throughout each experimental session and the entire procedure was repeated four times for two of the observers. The letter chart (three rows of ten 20/20 letters) was illuminated by an Argus projector which supplied sufficient illuminance to give an approximately five-degree square field and 910 trolands of retinal illuminance. All stray light was carefully screened from the subject's eyes. To insure that the subject focused on the letters and not the stigmatoscope light, a cam operated microswitch introduced between the optometer lights and the power supply flashed these lights on one second in every two.

Results

The mean data for each of the two subjects most extensively tested (they are typical in every way of all data obtained) are summarized in Figure 1. In this figure each plotted point is the mean of 12 independent determinations. For reasons which will become obvious, it is important to represent the data on three separate graphs.

The top graph shows the relation between accommodation response and accommodation stimulus. For intermediate levels the accommodation stimulus change effectively evoked an accommodation response. Over a wide range the relationship between the response and the stimulus was essentially a linear one. Like all other physiologic variables, however, the accommodation has a minimum and maximum value. As these limits were approached the response departed somewhat from linearity and when the stimulus change was greater than that necessary to evoke an extreme response in either direction, then either no further change in the response was evoked or a slight change in the wrong direction was elicited.

The middle graph in Figure 1 shows the concurrent vergence measurements as a function of the accommodation stimulus. For

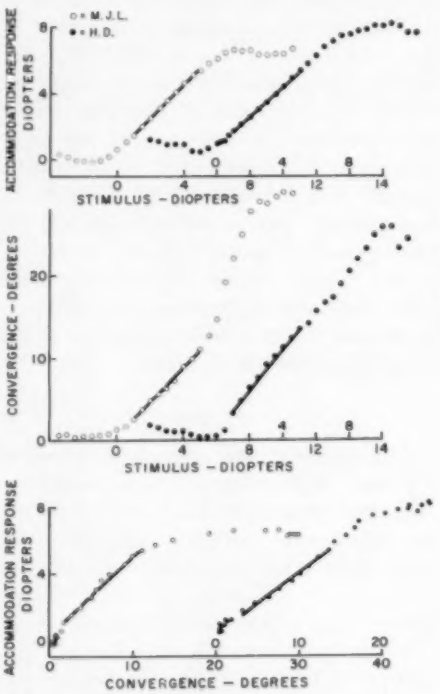

Fig. 1 (Alpern, Kincaid, and Lubeck). Results of the experiment for two observers. The plotted points are the means of 12 determinations obtained in four separate experimental sessions. The straight lines have been determined by the statistical procedures described in the text. (Top graph) Relation of accommodation response to accommodation stimulus. (Middle graph) Relation of the accommodative vergence to the accommodation stimulus. (Lower graph) Relation of accommodative vergence to accommodation response.

* The exact deviating power of this prism as measured at the center of rotation of the left eye was calibrated by two independent methods which closely agreed with each other. Such information is relevant only to the absolute value of the heterophoria and is of no consequence for the relative values (the concern of the present study) since the prism remained in the same position throughout all the measurements.

those stimulus values in which the accommodation response was linearly related to the stimulus, this graph shows that the vergence movement was also linearly related to the accommodation stimulus. At the lower limit of the accommodation stimulus the vergence reached a minimum value at approximately the same stimulus value as did the accommodation response and in this case also a further decrease in the stimulus evoked either no change in vergence or perhaps a slight movement in the opposite direction. At the upper limit of accommodation, however, an increase of the stimulus continued to be associated with an increase of vergence even when the stimulus change no longer was associated with an increase of accommodation. In fact, the stimulus evoked larger vergence movements here than at lower levels.

The lower graph shows the relationship between the two dependent variables (that is, accommodation response and vergence). Over the range in which the accommodation stimulus and response were linearly related, the relationship between accommodation response and vergence was also linear. This is an invariable finding in experiments of this kind. Outside this range, the relation between accommodation response and vergence also departed from linearity. As the two other graphs suggest, at upper levels the vergence continued to increase long after the accommodation response had reached a maximum value. Because of the current controversy in the literature over the relative linearity of such data as those in the bottom two graphs in Figure 1[5, 6] it should be emphasized that when the data are accumulated over a gamut of accommodation stimuli which is sufficiently large, none of these curves are ever linear.

Discussion

When an eye accommodates the two eyes converge and it is now proposed that the motor innervations to these two processes have a common point of origin in the central nervous system, that these innervations are linearly related to each other, and that they are evoked simultaneously by a change in accommodation stimulation. An example of evidence which supports this theory is the linearity of the relation of accommodation and vergence over the range of stimulation in which the accommodation response is linearly related to its stimulus and the lack of linearity beyond that range.

In the neighborhod of the near point of accommodation, it may be imagined that a unit change in accommodation stimulus still evokes a coresponding change in the accommodation innervation but owing to the sclerosis of the lens this innervation no longer evokes an accommodation response comparable to what can be obtained at lower levels. Figure 1 shows that at this limit of accommodation the vergence continued to change as the stimulus changed, and this would be explained as accommodative vergence associated with increase in the innervation to accommodation which failed to produce any further increase in accommodation because of the sclerosis of the lens.[7] The figures suggest that as the near point of accommodation was approached, a unit change in the accommodation stimulus evoked a greater change in the vergence than it did at levels in which the stimulus-response relation was linear. Within the present theoretical framework this effect can be interpreted as an increase in vergence which was associated with an increase in innervation to accommodation when the normal amount of innervation to accommodation elicited by a unit change in the stimulus failed to evoke the anticipated accommodation response. While it was a less common finding some of the results suggest that a similar (but much smaller) change in the relation between the stimulus to accommodation and the magnitude of motor innervation may occasionally occur in the neighborhood of the far point of accommodation.*

* The admittedly rare case in which nonlinear relations between accommodation and vergence have been described[5, 8] can probably be best explained as

In order to quantify this relationship between accommodation and vergence it is best to state the theory in algebraic form.

We introduce the following notation:

u = magnitude of central motor innervation
X = accommodation stimulus
Y_a = measured accommodation response
Y_c = measured accommodative vergence
γ_u = physiologic variation in central innervation
γ'_a = physiologic variation in accommodation
γ'_c = physiologic variation in accommodative vergence
γ''_a = error in measurement of accommodation
γ''_c = error in measurement of accommodative vergence
m_u, m_{au}, m_{cu}, k_u, k_{au}, k_{cu} are constants.

The quantities γ_u, γ'_a, and so forth, may be regarded as random variables having zero mean values.

The basic theoretic position (stated above) may also be expressed by the following three equations:*

$$u = m_u X + k_u + \gamma_u, \tag{1}$$

$$Y_a = m_{au} u + k_{au} + \gamma'_a + \gamma''_a, \tag{2}$$

$$Y_c = m_{cu} u + k_{cu} + \gamma'_c + \gamma''_c. \tag{3}$$

From the foregoing relations, we see that m_{au} and m_{cu} represent the mean changes in accommodation and vergence, respectively, induced by a unit change in central innervation. Thus the ratio m_{cu}/m_{au} may be regarded as the ratio between innervation to vergence change and innervation to accommodation. This ratio will be designated as the *response AC/A ratio*.

Combining (1) and (2) we obtain the relation

$$Y_a = m_a X + k_a + \gamma_a, \tag{4}$$

where

$$m_a = m_{au} \cdot m_u,\ k_a = m_{au} k_u + k_{au}, \gamma_a = m_{au}\gamma_u + \gamma'_a + \gamma''_a. \tag{5}$$

Similarly, we get from (1) and (3)

$$Y_c = m_c X + k_c + \gamma_c, \tag{6}$$

with

$$m_c = m_{cu} \cdot m_u,\ k_c = m_{cu} k_u + k_{cu},\ \gamma_c = m_{cu}\gamma_u + \gamma'_c + \gamma''_c. \tag{7}$$

The relations (4) and (6) state that measured accommodation and accommodative vergence are linearly related to the accommodative stimulus, apart from physiologic variability and errors of measurement; thus (4) and (6) correspond to the experimentally observed relationships between these variables.

Combining (4) and (6) yields a linear relation between Y_a and Y_c:

$$Y_a = \frac{m_a}{m_c} Y_c + k_a - \frac{m_a}{m_c} k_c + \gamma_a - \frac{m_a}{m_c} \gamma_c. \tag{8}$$

From (5) and (7) we get

$$\frac{m_c}{m_a} = \frac{m_{cu} m_u}{m_{au} m_u} = \frac{m_{cu}}{m_{au}}, \tag{9}$$

which shows that the response AC/A ratio is the reciprocal of the coefficient of Y_c in (8). In geometric terms, this means that the response AC/A ratio is the reciprocal of the slope of a line that represents the relationship between Y_a and Y_c apart from random fluctuations. Thus one might hope to approximate this ratio by fitting a line to a set of points plotted as in Figure 2C.

The usual least-squares procedure for fitting such a line is not appropriate in this case, however, since both Y_a and Y_c, that is, both co-ordinates of the plotted points, are assumed subject to random influences.

Another possibility is to minimize the sum of the squares of the perpendicular distances between the points and the line. While this approach yields a reasonably well-fitting line, it suffers from the drawback that the perpendicular distance has no natural interpretation since the variables are not geometric distances. Indeed, the line obtained depends on the units in which the variables are measured.

At this point we may recall that the values of the stimulus X corresponding to the observed values of Y_a and Y_c also form part of the data. In view of the relations (4) and

measurements made in which the stimulus to accommodation values were not confined to the range in which the accommodation stimulus-response curve is linear, although another explanation equally compatible with the above theory has also been made.[4] A crucial result *not* compatible with the theory would be the demonstration of a curvilinear relation between accommodation and vergence for a series of stimulus to accommodation values within the range in which the relation between accommodation stimulus and accommodation response is linear. Repeated attempts in this laboratory have failed to demonstrate any clear-cut example of this in any of the subjects so far examined.

(6), the response AC/A ratio might be determined by fitting straight lines to the plots of Y_a against X and Y_c against X and then using (9).

Since the errors in measurement of X may safely be neglected, it would seem appropiate to apply the usual least-squares criterion in fitting these lines. However, it is apparent from (5) and (7) that the random components γ_a and γ_c of Y_a and Y_c are not in general independent of each other, and one may wonder whether this lack of independence should be taken account of in choosing the best fitting lines.

To resolve this question, it is useful to recall that the least-squares criterion may be regarded as a special case of the maximum likelihood principle. That is, if one assumes (in the usual curve-fitting situation with one independent and one dependent variable) that random variations in the dependent variable are normally distributed with uniform variance, one can compute the re'ative probability that a repetition of the experiment will result in a repetition of the data if the mean value of the dependent variable is a specified function of the independent variable. This probability, referred to as the likelihood of the observed data, varies inversely with the sum of the squares of the deviations of the experimentally obtained values of the dependent variables from the mean values predicted by the function, and thus the likelihood will be a maximum when the sum of squares is a minimum. The maximum likelihood principle is also applicable to more general situations, however, in which it may not correspond to the least-squares criterion. Our next step, therefore, will be to apply the maximum likelihood principle to the present problem.

Let us assume that the random deviations γ_a and γ_c have a joint normal distribution with zero means, standard deviations σ_a and σ_c, and correlation ρ; that is, that they have the joint frequency function

$$f(\gamma_a, \gamma_c) = \frac{1}{2\pi\sigma_a\sigma_c\sqrt{1-\rho^2}} e^{-Q}, \tag{10}$$

with

$$Q = \frac{1}{2(1-\rho^2)}\left(\frac{\gamma_a^2}{\sigma_a^2} - \frac{2\rho\gamma_a\gamma_c}{\sigma_a\sigma_c} + \frac{\gamma_c^2}{\sigma_c^2}\right). \tag{11}$$

The likelihood to be maximized is the product

$$L = \prod f(\gamma_a, \gamma_c) = \prod f(Y_a - m_aX - k_a, Y_c - m_cX - k_c), \tag{12}$$

taken over the set of values of X for which observations were taken. In view of (10) and (11) maximizing L is equivalent to minimizing the sum

$$L' = \sum\left[\frac{(Y_a - m_aX - k_a)^2}{\sigma_a^2} - \frac{2\rho(Y_a - m_aX - k_a)(Y_c - m_cX - k_c)}{\sigma_a\sigma_c} + \frac{(Y_c - m_cX - k_c)^2}{\sigma_c^2}\right]. \tag{13}$$

It is apparent that if $\rho = 0$ this is equivalent to selecting the constants m_a, k_a, m_c, and k_c in accordance with the least-squares criterion, which means that they satisfy the conditions

$$\begin{aligned} &\sum (Y_a - m_aX - k_a) = 0, \quad \sum X(Y_a - m_aX - k_a) = 0, \\ &\sum (Y_c - m_cX - k_c) = 0, \quad \sum X(Y_c - m_cX - k_c) = 0. \end{aligned} \tag{14}$$

It is not obvious that the same conditions determine the minimum when $\rho \neq 0$. However, we shall show that this is the case.

Let m_a^*, k_a^*, m_c^*, and k_c^* be values of the parameters satisfying the conditions (14). Then (13) may be put in the form

$$\begin{aligned} L' = \sum\Bigg[&\frac{(Y_a - m_a^*X - k_a^* + (m_a^* - m_a)X + k_a^* - k_a)^2}{\sigma_a^2} \\ &- \frac{2\rho(Y_a - m_a^*X - k_a^* + (m_a^* - m_a)X + k_a^* - k_a)(Y_c - m_c^*X - k_c^* + (m_c^* - m_c)X + k_c^* - k_c)}{\sigma_a\sigma_c} \\ &+ \frac{(Y_c - m_c^*X - k_c^* + (m_c^* - m_c)X + k_c^* - k_c)^2}{\sigma_c^2}\Bigg]. \end{aligned}$$

* All of these equations are valid over the range of values of X for which the relationship between Y_a and X is linear. Beyond this range (2) is no longer valid. In individual cases (1) and (3) may continue to be valid outside this range.

Expanding the first term of (15), one obtains the expression

$$\frac{1}{\sigma_a^2}\sum\{(Y_a-m_a^*X-k_a)^2 +2(Y_a-m_a^*X-k_a^*)[(m_a^*-m_a)X+k_a^*-k_a] +[(m_a^*-m_a)X+k_a^*-k_a]^2\},$$

of which the middle term drops out since the starred parameters satisfy (14). The other terms of (15) may be evaluated similarly, leaving

$$\begin{aligned}L'=\sum\{&\frac{1}{\sigma_a^2}(Y_a-m_a^*X-k_a^*)^2\\ &-\frac{2\rho}{\sigma_a\sigma_c}(Y_a-m_a^*X-k_a^*)(Y_c-m_c^*X-k_c^*\\ &+\frac{1}{\sigma_c^2}(Y_c-m_c^*X-k_c^*)^2\\ &+\frac{1}{\sigma_a^2}[(m_a^*-m_a)X+k_a^*-k_a]^2\\ &-\frac{2\rho}{\sigma_a\sigma_c}[(m_a^*-m_a)X+k_a^*-k_a\\ &\cdot[(m_c^*-m_c)X+k_c^*-k_c]\\ &+\frac{1}{\sigma_c^2}[(m_c^*-m_c)X+k_c^*-k_c]^2\}.\end{aligned} \tag{16}$$

Of the terms following the summation symbol in (16), the last three indicate the effect of changing the parameters. They may be rewritten in the form

$$\begin{aligned}&\frac{1}{2}(1+\rho)\left[\frac{(m_a^*-m_a)X+k_a^*-k_a}{\sigma_a}-\frac{(m_c^*-m_c)X+k_c^*-k_c}{\sigma_c}\right]^2\\ &+\frac{1}{2}(1-\rho)\left[\frac{(m_a^*-m_a)X+k_a^*-k_a}{\sigma_a}+\frac{(m_c^*-m_c)X+k_c^*-k_c}{\sigma_c}\right]^2,\end{aligned} \tag{17}$$

which is clearly positive for $-1 \leq \rho \leq 1$ unless $m_a = m_a^*$, $k_a = k_a^*$, and so forth. This demonstrates our assertion that the minimum value of L′ occurs when the parameters satisfy the least-squares conditions (14), and thus shows that it is reasonable to determine the response AC/A ratio in the manner suggested.

Figure 2 illustrates the results of the application of the procedures outlined above for the determination of linear equations for the data obtained over the range $1.00 \leq X \leq 5.00$ for observer M. J. L. In this figure each plotted point is an individual datum.

We have followed this procedure for a wide variety of stimulus conditions and a large number of observers and have invariably found the lines constructed to be a satisfactory representation of the data.

In clinical practice it is not possible to determine the value for the response AC/A ratio since the magnitude of the change in accommodation as the stimulus is changed cannot easily be determined. In this case, it is necessary to rely upon the magnitude of vergence associated with a unit change in the stimulus to accommodation. This, of course, is simply m_c. It is proposed to refer to this quantity as the *stimulus AC/A ratio.* How closely can one estimate the response AC/A ratio once he has succeeded in determining the stimulus AC/A ratio? Since the value of m_a will usually be only slightly smaller than unity, it is to be expected that the former will be but slightly larger than the latter. Indeed an analysis of some 37 measurements made in this way indicates that the response AC/A may reasonably be predicted by multiplying the stimulus AC/A by 1.08. The comparison is illustrated in Figure 3. These data suggest that considerable clinical usefulness can be anticipated from the determination of the stimulus AC/A. It is recognized that the procedures utilized for the evaluation of m_c are still much too cumbersome to permit any clinical application but the methods proposed in this present study are regarded as a necessary step in the establishment of the validity of clinically more practical procedures. Future experiments will be devoted to attempts to establish this validity.

The present paper has provided operational definitions of the AC/A ratios. It sheds no light at all on the answers to a number of clinically and theoretically important questions. How important a characteristic of individual observers are the AC/A ratios? Can the response and stimulus AC/A ratios be modified to any degree by practice effects? What is the relation between AC/A

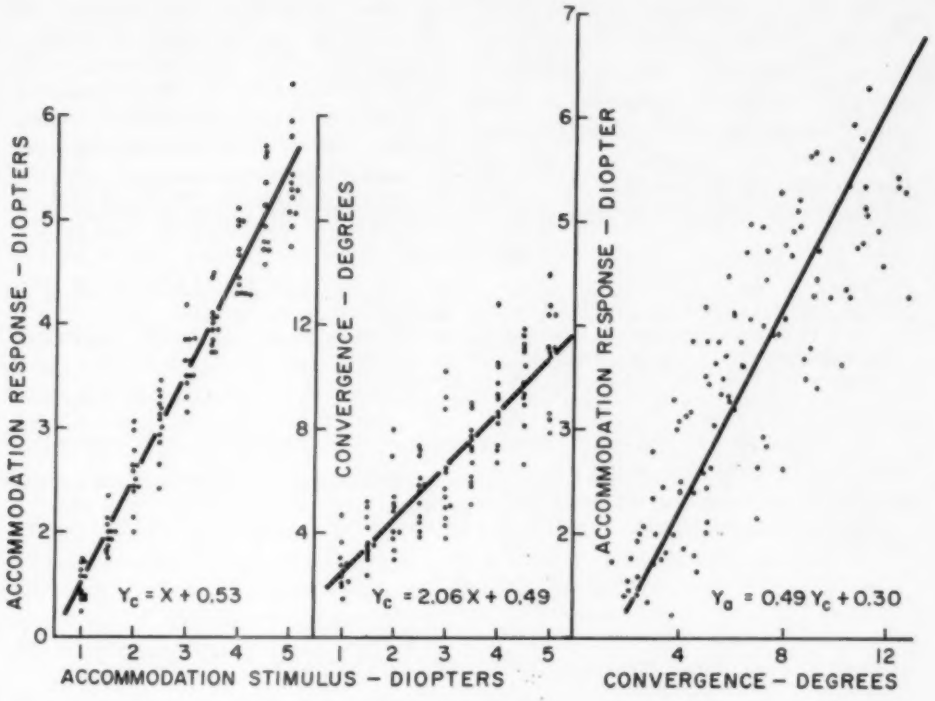
ACCOMMODATION RESPONSE – DIOPTERS
$Y_c = X + 0.53$
ACCOMMODATION STIMULUS – DIOPTERS
CONVERGENCE – DEGREES
$Y_c = 2.06X + 0.49$
ACCOMMODATION RESPONSE – DIOPTER
$Y_a = 0.49 Y_c + 0.30$
CONVERGENCE – DEGREES

Fig. 2 (Alpern, Kincaid, and Lubeck). Individual determinations for observer M. J. L. in the range ($1.00 \leq \times \leq 5.00$) in which the relation between accommodation stimulus and response is linear. (A) The left-hand graph shows the relation between accommodation stimulus and response. (B) The middle graph shows the relation between accommodative vergence and accommodation stimulus. (C) The right-hand graph shows the relation between accommodation response and accommodative vergence.

ratios as defined in this paper and measurements made with the fixation disparity technique? What are the limits of fluctuation of the AC/A ratios in a given observer from moment to moment and from day to day? What are the effects of a wide variety of variables such as drugs, age, and sex on these quantities? All of these questions are now open to straightforward experimental solution, however, since operational definitions of these quantities have been given.

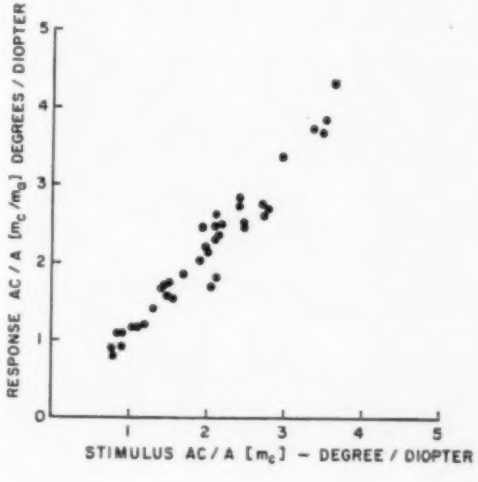

Fig. 3 (Alpern, Kincaid, and Lubeck). Scatter plot showing the relation between stimulus AC/A and response AC/A, as measured on eight young adult observers.

SUMMARY

Haploscopic measurements of accommodation and accommodative vergence over a wide range of stimuli to accommodation were carried out on four young adult observers. The data can be quantified by introducing two concepts: (a) the response AC/A ratio and (b) the stimulus AC/A ratio. A theoretic explanation of the synkinesis between accommodation and accommodative vergence is proposed which is based on the notion that motor innervations to the two systems have a common point of origin within the central nervous system. Procedures are developed for estimating these two ratios.

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THE ELICITATION OF FUSION IN STRABISMUS PATIENTS WHO ARE AWARE OF DIPLOPIA*

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In a previous paper,[1] an experiment was described in which 59 strabismus patients were made temporarily aware of diplopia and were then taught to alternate back and forth repeatedly between right-eye fixation and left-eye fixation. This was accomplished with the aid of special apparatus. These patients then practiced alternation with diplopia as a daily home exercise for periods of from two weeks to one year. The effect of this was to make 50 of the 59 patients (85 percent) measurably more aware of diplopia. Twenty-five patients (42 percent) became acutely aware of diplopia. So far as could be determined on clinical examination, these 25 patients were incapable of monocular suppression.

On the basis of certain theoretic arguments, it was deduced that patients who were incapable of monocular suppression might become capable of binocular fusion if they continued to practice this exercise of alternation with awareness of diplopia. The original group of 59 patients had been under treatment at the Kresge Eye Institute in Detroit. Since I am no longer associated with that institute, the continued treatment of those patients under my management has not been feasible. Nevertheless, some evidence is available which bears on the question of the effectiveness of the alternation exercise in eliciting fusion. The purpose of this paper is to present that evidence.

* From the Department of Ophthalmology, University of Michigan. Read before the East-Central Section, Cincinnati, Ohio, January 5, 1959. This research was supported in part by the Knights Templar Eye Foundation, Inc.

Method

Figure 1 shows the two images of a single object—a coffee cup—as they appear to a patient with homonymous diplopia. If the patient is fixating the object with his right eye (upper portion of the figure), he sees the right-eye image in the correct place, or straight ahead. At the same time, he sees the left-eye image—the squinting-eye image—off to the left. If the patient is fixating the object with his left eye (lower portion of the figure), he sees the left-eye image straight ahead and the right-eye image off to the right. If the patient can remain aware of diplopia when he alternates from right-eye fixation to left-eye fixation, he should see the two images move to the right. Similarly, on alternating from left-eye fixation to right-eye fixation, he should see the two images move to the left.

This phenomenon of apparent movement of images on alternation of fixation is an essential feature of the alternation exercise and also provides an effective way of teaching patients to perform that exercise. For example, suppose we have an esotrope who is aware of diplopia but who cannot perform the exercise because he is unable to fixate with his left eye. In order to teach him to fixate with his left eye, we first call his attention to the fact that he sees the right-eye image of any object straight ahead and the left-eye image of the same object off to the left. Then we cover his right eye, and have him note that when we do so the left-eye image becomes straight ahead. Next, by momentary uncovering of the right eye, we teach him to see the right-eye image off to the right while the left-eye image is still straight ahead. The rest is a matter of progressive training. The patient learns to keep the left-eye image straight ahead while the right eye is uncovered for longer and longer periods. When he can do this for 10 seconds, he can then learn to alternate from right-eye fixation to left-eye fixation by "making the left one straight ahead." From the point of view of the patients to be described in this report, the exercise which they practiced was not repeated alternation of fixation. It was "making the two images go back and forth without losing either one."

Fig. 1 (McLaughlin). Two images of a single object as they appear to a patient with homonymous diplopia.

When a patient reports that he sees the left-eye image straight ahead, he will be found to be fixating with his left eye. When a patient reports that he sees the two images move back and forth as illustrated in Figure 1, he will be found to be alternating back and forth repeatedly between right-eye fixation and left-eye fixation.

The key feature of the exercise is that the patient must learn to see the two images actually moving back and forth on each alternation of fixation. Not only should the images be seen to move, but neither image should be allowed to disappear even for an instant at any time during the exercise. Both should be seen at all times. No patient has become capable of fusion who had not perceived this movement of images when performing the exercise.

In the absence of specific instructions to the contrary, many strabismus patients who perform the exercise correctly in terms of alternation of fixation and in terms of awareness of diplopia, nevertheless report that only one image—the squinting -eye image—moves during the exercise. These patients describe the squinting -eye image as moving from side to side of the fixing-eye image on each alternation of fixation. This appearance is not at variance with the localization phenomena illustrated in Figure 1. Nevertheless, from the point of view of the patient's subjective experience, it is a different visual ex-

ercise than "making the two images go back and forth." All patients described in this report, and all patients under the writer's management, have been specificially instructed to perceive both images as moving concomitantly, and have succeeded in doing so. The rationale of this procedure is that the exercise is effective in aiding binocular fusion only if it causes the patient to perceive each monocular image as alternately "straight ahead" and "off to the side." This argument will be stated more fully in the theoretic section of this paper.

The foregoing method of teaching patients to perform the exercise is effective with patients who have a good awareness of diplopia. Methods for use with patients who are not aware of diplopia have been described in a previous report.

Prior to the development of these "apparent movement" methods of instruction, the writer was able to teach the alternation exercise to only a few selected patients. It is now possible to teach over 90 percent of patients to perform the exercise correctly.

When a patient had learned to perform the exercise correctly, he was advised to do so for 15 minutes each day. Patients were encouraged to use ordinary household objects rather than special test objects in performing the exercise. Patients were examined weekly or biweekly for ability to perform the exercise correctly.

The term "fusion" in the present report refers to the subjective unification of the two monocular images of an object which subtends less than one degree of visual angle at a viewing distance of six meters, combined with simultaneous binocular foveal fixation of that object. The criteria of fusion were (a) absence of diplopia and (b) absence of observable deviation on applied cover. It sometimes happened that a patient could not fuse on command, but occasionally fused involuntarily. Such a patient was considered able to fuse involuntarily for purposes of the present investigation, the only requirement here being that the patient be able to fuse long enough for the applied cover test to be carried out with each eye in turn.

By means of repeated extensive examinations, particular care was taken to ensure that patients were not able to fuse at the start of treatment.

Results

Of the original group of 59 patients, five had intermittent tropias at the start of treatment. Of the remaining 54, seven (13 percent have become capable of fusion with no other treatment than continued performance of the alternation exercise. The writer has been informed that nine additional patients under treatment at the Kresge Eye Institute, not from the original group of 59 patients, have achieved a similar result. These nine patients were managed by the orthoptic staff of the Kresge Eye Institute, and in each instance the capacity for fusion was obtained with no other treatment than alternation with diplopia.

Of the 30 patients now under my management at the University of Michigan, only one has been under treatment for more than 25 weeks. Two patients out of the 30 have become capable of fusion with no other treatment than alternation with diplopia.

To the best of my knowledge, the capacity for fusion has not been elicited by this method in any patient having a vertical tropia of more than two prism diopters, nor in any patient having a visual acuity difference between the two eyes of more than three lines on the Snellen chart. Binocular fusion has been elicited in both male and female patients, ranging in age from five years five months to 15 years two months.

More than 20 patients have discontinued treatment for personal reasons after having become aware of diplopia. None of these patients has so far become capable of fusion.

No patient who has been made aware of diplopia by means of the method described here has yet reported any visual discomfort

or confusion associated with the awareness of diplopia. About 200 patients have been made aware of diplopia by this means.

Case reports

Case 1

A white boy, aged 10 years 10 months at the start of treatment. Visual acuity: O.D., 20/15; O.S. 20/40 (Snellen). Prism and cover findings were exotropia of eight prism diopters at six m., esotropia of four prism diopters at 33 cm., with correction. Retinoscope findings under cycloplegia were: O.D., +1.25D. sph. ⊃ +1.5D. cyl. ax. 97°; O.S., +2.0D. sph. ⊃ +0.75D. cyl. ax. 56°. Onset of strabismus was prior to nine months of age. Three courses of orthoptic therapy were given without success; the latest terminated in 1956. There was no awareness of diplopia under ordinary conditions of viewing. The patient fixated with the right eye but could not hold the left-eye fixation.

This patient was taken under treatment in January, 1958. He learned on first examination to perform the alternation exercise with the aid of special apparatus. Within one month, he learned to perform the same exercise with ordinary objects. During the next six months, the patient repeatedly reported that he was able to "bring the two images together" but examination revealed that he was then achieving single vision by monocular suppression rather than by binocular fusion. During the same period, the patient repeatedly regressed so that he was unable to perform the alternation exercise, and had to relearn it.

By August, 1958, this patient's awareness of diplopia had increased to the extent that he was apparently incapable of monocular suppression. In October, 1958, he was capable of binocular fusion at six m. and at 33 cm. He is still under treatment, and is still performing the same exercise. The purpose of the present treatment is to attempt to elicit habitual fusion.

Case 2

A white girl, aged 13 years one month. Onset was before the first year of age. No previous orthoptic or surgical treatment had been given. Vision: 20/20 Snellen either eye, without correction. Prism and cover findings were 28 prism diopters of exotropia at six m., and 33 prism diopters of exotropia at 33 cm. There was no history of fusion and no knowledge of diplopia.

This patient learned on first examination to perform the alternation exercise with the aid of special apparatus. At the third weekly examination, the patient learned to perform the exercise with ordinary objects. She manifested spontaneous fusion at the third examination. Same therapy was continued. No convergence exercises were given and no minus lenses were used. Near-point of convergence was remote on first examination, and to nose after three months of treatment. At that time the patient was habitually fusing so far as could be determined by clinical examination. After nine months of treatment, prism and cover measurements had not changed from first examination, in spite of the fact that the patient had difficulty in performing the alternation exercise due to a strong tendency to fusion. She was then discharged, to be seen in three months.

Case 3

A white boy, aged five years five months. Onset was before the age of three and one-half years. There was small accommodative esotropia, less than 12 prism diopters at near and five at far without correction and equal visual acuity with correction. A course of orthoptic therapy had been unsuccessful.

The chief difficulty with this patient, as with others in his age group, was to convey the notion of simultaneous binocular perception. This was done successfully after five weeks of treatment. He showed capacity for binocular fusion 17 weeks from the start of treatment. The patient has now been under treatment for 22 weeks.

Cases 1 and 3 are under treatment at the University of Michigan. Case 2 was one of the original 59 patients treated at the Kresge Eye Institute and described in a previous report.[1] Case 2 was managed, during the first month of treatment, by Miss Arlene Stearns, head of the Orthoptic Department of the Kresge Eye Institute.

The most striking common characteristic of these three patients is that all three were unusually co-operative and diligent in the performance of home exercises. This has been a characteristic of every patient in whom a positive result has been obtained.

Theoretic discussion

In the upper portion of Figure 1, the right-eye image correctly represents the position of the coffee cup, and the left-eye image does not. The chief source of visual confusion in diplopia must be an inability of the observer to decide, quickly and accurately, which image is the correct one.

If an individual is aware of diplopia but is not confused or annoyed by that awareness, we must infer that he is able to differentiate, reflexly and accurately, between the two images of any object: he must know without thinking about it which one is correct and

which one is incorrect. Of more than 200 strabismus patients who have been examined and interrogated on this point, only four manifested any confusion or discomfort associated with their awareness of diplopia. Those four were adult patients with paralytic strabismus of recent onset. It follows that the ability to identify fixing-eye and squinting-eye visual images as such, and to differentiate between them, is a general characteristic of strabismus patients who are aware of diplopia.

In another paper[1] I have presented evidence that this reflex differentiation between fixing-eye images and squinting-eye images is not only the means by which strabismus patients avoid confusion when they are aware of diplopia, but is also the mechanism of monocular suppression in strabismus. In other words, it is the means by which strabismus patients ordinarily avoid being aware of diplopia.

There are undoubtedly many factors both motor and sensory, which inhibit fusion in strabismus. One of the sensory factors may be that the two monocular visual images of any object are reflexly perceived in two very different ways, as described above: one is perceived as an image, the other as a real object. In this view, one reason the patient does not fuse the two images is that for him they do not belong together. One represents a solid, tangible object; the other represents only itself.

In order to understand this hypothesis, one must appreciate that the real-unreal distinction which the patient makes between fixing-eye and squinting-eye visual images is a far more fundamental differentiation than, for example, a distinction between bright and dark images, or between clear and blurred images. The real-unreal distinction in strabismus is a differentiation between things that exist and things that do not exist, between the tangible and the intangible. From this point of view, the hypothesis that the real-unreal differentiation may inhibit fusion in strabismus appears to merit experimental evaluation.

Fig. 2 (McLaughlin). The two images of a single object (a coffee cup) as they appear to a patient with homonymous diplopia. Shading of the squinting-eye images is intended to represent the perceptual attribute of unreality which those images have for the patient.

In view of these considerations, an effort has been made to treat strabismus patients by making it difficult for the patients to maintain that differentiation between fixing-eye and squinting-eye visual images. More specifically, the rationale of therapy which has been employed here is to have the patient practice a visual exercise which causes him to perceive both images as equally "real" at the same time.

This is illustrated in Figure 2, which is similar to Figure 1 except that the squinting-eye image has been shaded. This shading is intended to represent the special quality of unreality which the patient perceives in the squinting-eye image. If the patient remains aware of diplopia while he alternates back and forth, each image taken alone will be perceived as alternately "real" and "unreal." This is a highly anomalous appearance, and the patient who performs this exercise correctly will describe it as highly anomalous. Nothing else that the patient ever sees changes back and forth repeatedly between being a real object and being an image. Hence the effect of prolonged practice of the exercise should be to weaken the reflex differentiation between the two images.

It has previously been shown that strabismus patients can be made progressively more aware of diplopia by means of the alternation exercise described here. That finding lends support to the hypothesis just stated. The present finding, that alternation with

diplopia can aid binocular fusion in some strabismus patients, further substantiates the view that a reflex differentiation between the two monocular images of any object is a salient feature of binocular visual perception in strabismus.

3536 Kresge Building.

Reference

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STUDIES ON THE VISUAL TOXICITY OF METHANOL*

X. Further observations on the ethanol therapy of acute methanol poisoning in monkeys

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In previous publications we have described how methanol poisoning in monkeys and humans represents a disease entity, and differs biochemically and clinically from the effect of methanol on subprimates.[1] We have also described how repeated doses of ethanol, begun at the time the single dose of methanol is given, prevent eye damage, acidosis, and death in monkeys.[2]

This ideal therapeutic situation never occurs in human methanol poisoning—although the issue is often confused by the initial ingestion of mixtures of ethanol and methanol. It is therefore desirable to know how long ethanol therapy may be delayed and still be effective. This was the purpose of the monkey experiments reported here.

The pertinent literature was reviewed in the previous report.[2] Additional papers of interest are those of Strittmater and Ball[3] and of Kinoshita and Masurat[4] who reported a specific formaldehyde dehydrogenase in liver and in retina, respectively. Whether this is the sole significant path of formaldehyde oxidation in vivo is a point of considerable interest, but this is as yet uncertain. Hence its significance for methanol poisoning cannot be assessed.

Materials and Methods

The plan for our present group of experiments was to give monkeys a single oral dose of 6.0 gm./kg. of methanol. Ethanol therapy was to be started at varying time intervals after the poisoning. Surviving monkeys were to be allowed to recuperate for a minimum of three weeks and then be given the same dose of methanol without treatment. To date, we have a total of nine monkeys getting 6.0 gm./kg. of methanol, untreated; of the nine, seven died (78 percent).

Most of the experimental details have been previously described.[1,2] Only changes made in the present study are here reported.

Monkeys

The shortage of rhesus monkeys resulting from the poliomyelitis vaccine program forced us to change monkey species before these experiments were completed.

The species to which we shifted was the guenon (grivet monkey), Cercopithecus aethiops aethiops.[5] These animals showed the typical clinical picture of methanol poisoning as described for the rhesus monkey, including the profound acidosis.

In only one detail did there seem to be a difference between rhesus and guenon: this was in the CO_2 combining capacity levels for normal plasmas.

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Mathematical analysis suggests the possibility that normal plasma CO_2 combining capacities are higher in guenons than in the rhesus monkeys ($0.02 > P > 0.01$); we are comparing only eight guenons against 34 rhesus macaques.

One rhesus monkey had diarrhea and a second had anuria which, we feared, obscured the validity of the biochemical data. In addition one guenon showed moderate diffuse pulmonary edema at death. Hence biochemical data on these three animals were omitted. Since, however, these intercurrent findings could not conceivably increase survival time, it was considered proper to include survival data on the animals in question.

Clinical management

All monkeys on delayed ethanol therapy had no food, but did have water *ad lib.*, during the 24 hours preceding administration of methanol. This was done to promote uniformity of gastrointestinal absorption. Monkeys were watched constantly for two or more hours after methanol administration so that we could have measurements of any significant amounts of methanol lost by vomiting. In control experiments, water was given every eight hours equal in volume to the fluids given during the same period of time in the ethanol-treated experiments.

Methods

Methanol levels are reported only on samples in which the following procedure was carried out: Bloods were centrifuged and the plasmas frozen in less than one hour after collection. The plasma was kept constantly frozen except when samples were removed for purpose of analysis.

Our standard method for methanol determination was a modification of the chromotropic acid method of Bricker and Johnson.[6] In three experiments (Elagabalus, Epandamanas, and Esau in their control, untreated experiments) methanol levels were determined by the microdiffusion method of Sunshine and Nenad.[7] Experiments on plasma containing known amounts of methanol showed that the Sunshine method gave values high by a factor of 1.35. In other words, the potassium bichromate used in this method oxidizes methanol beyond the formaldehyde stage, and more than one equivalent of bichromate was utilized per equivalent for alcohol. Our reported methanol values in these three experiments are therefore the result of dividing the experimental values by 1.35.

Ethanol levels were calculated by determining the total alcohol level by the Sunshine method and from this value the amount of methanol, obtained by the chromotropic acid method and multiplied by a factor of 1.35, was subtracted.

Results

A. Clinical

This batch of monkeys generally followed the course of monkey methanol poisoning as described by us earlier. Their clinical course resembled that of human poisoning. About 80 percent of the untreated monkeys at this dosage level progress to eye and brain damage, peripheral vasomotor shock, coma, and death.

This process was reversible when ethanol treatment was delayed as long as eight and 12 hours. See Table 1 for a summary. The monkey having a 12-hour delay in treatment was barely able to pull through. With 16- and 20-hours' delay before starting ethanol, the monkeys died. (One 20-hour monkey was "resistant," being able to survive almost three times the minimal lethal dose without treatment.)

Although we could not save the monkeys after a 16 hour delay in ethanol therapy, we were able to prolong their lives. These results are shown in Table 2. The number of untreated monkeys comprises our total experience to date at this dosage level; six of them have been reported previously.[1,2,8] One of the untreated monkeys had a lower methanol dosage, 5.0 gm./kg. He is included because a lower methanol dose should lengthen the time of death; his death at 26 hours would therefore not unjustly weight our statistics. Our data show a significant increase in length of life in the group on ethanol therapy as compared to those without treatment.

Four of our five treated monkeys showed no eye changes. See Table 1. The fifth monkey started ethanol 20 hours after poisoning; he showed bilateral minimal retinal edema and inequalities in pupillary size, starting at 48 hours and lasting until death. In three experiments without ethanol therapy only one monkey showed eye abnormalities. These were bilateral hyperemia of the discs, blurring of the disc margins, retinal edema, and absent pupillary responses to light. All of these changes were first noted four hours before death. Microscopic examination of the eyes of the monkey getting ethanol treatment showed no departure from normal controls.

TABLE 1

CLINICAL EFFECTS AND DOSAGE OF DELAYED ETHANOL THERAPY OF ACUTE METHANOL POISONING

Monkey	Species	Body Weight (kg.)	Time Since First Experiment (days)	Dose 20% MeOH (gm./kg.)	EtOH Started (hr. after poisoning)	EtOH # of Doses Given Every 4 Hr.	EtOH Amount Each Dose (gm./kg.)	EtOH Time Last Dose (hr. after poisoning)	Eye Examinations*	Clinical Results
1(A) Dionysus	Rhesus	3.0		6.0	8	1 2–16	0.75 0.50	68	Normal	Survived
(B) Dionysus	Rhesus	3.4	48	6.0					O.U., Hyperemia of discs, blurring disc margins, retinal edema, absent pupillary responses to light	Died at 28 hr.
2(A) Dalton	Grivet	2.3		6.0	12	1–5 6–17	1.0 0.5	76	Normal	Survived
(B) Dalton	Grivet	2.6	30	6.0					Normal	Died 30–35 hr.
3 Democritus	Grivet	2.4		6.0	16	1–6 7–8	1.0 0.5	44	Normal	Died at 48 hr.
4(A) Domitian	Rhesus	3.3		6.0	20 60	1 2–8 9 10–15	0.75 0.50 0.75 0.50	52 84	Normal	Survived
(B) Domitian	Rhesus	3.5	21	6.0					Normal	Survived
5 Darwin	Grivet	4.4		6.0	20	1–16	1.0	80	O.U., Early retinal edema; variable pupillary inequalities in size	Died 90–91 hr.

* Pupillary and corneal changes associated with coma are not reported.

TABLE 2

EFFECT OF DELAYED ETHANOL THERAPY ON TIME OF DEATH CAUSED BY 6.0 GM. METHANOL/KG. MONKEY

No Treatment			Delayed Ethanol Treatment			
No.	Monkey	Time of Death (hr. after MeOH)	No.	Monkey	Time EtOH Started (hr. after MeOH)	Time of Death (hr. after MeOH)
1	Erebus	17	1	Dillinger	10	83–88
2	Esau	18–19	2	Dagon	12	117–136
3	Short Stop	20	3	Democritus	16	48
4	Elpenor*	26	4	Darius	20	37–40
5	Dionysus	28	5	Darwin	20	90–91
6	Pain in the Neck	30				
7	Dalton	30–35				
8	Epandamanas	36–38				

* Had only 5 gm. MeOH/kg.

	No. Monkeys Dead <36 Hours after MeOH	No. Monkeys Dead >36 Hours after MeOH	Total
No Treatment	7	1	8
Delayed EtOH Rx	0	5	5
Total	7	6	13
Exact Probability = 0.0047			

The varying doses of ethanol are detailed in Table 1. In our early experiments we used the dosage schedule we have found to be the minimum optimal in our monkeys starting treatment at the time of poisoning.[2] When this proved ineffectual in saving lives after longer than 12 hours' delay, we roughly doubled the dose but found no increased effectiveness. We did not further increase the ethanol dosage. Continued increases would ultimately lead us to the level at which we could expect increased mortality from the additive narcotic effects of the two alcohols. Monkey No. 5 received 1.0 gm./kg. at every dose. With monkeys No. 2A and No. 3 the early doses were 1.0 gm./kg. This was dropped to 0.5 gm./kg. when their clinical conditions seemed perhaps to be worsening rather than improving.

Clinically no clear-cut difference in immediate response to 1.0 or 0.5 gm./kg. ethanol was noted. This lack of obvious distinction was probably due to the fact that the methanol poisoning overshadowed signs from the much smaller doses of ethanol.

Plasma ethanol levels could provide a check on the effectiveness of therapy. Unfortunately, potassium bichromate oxidizes methanol beyond the aldehyde stage, and the determination of small quantities of ethanol in the presence of large quantities of methanol was highly unreliable. Thus plasma ethanol levels by our methods could not be utilized for the above purpose.

B. Plasma carbon-dioxide combining capacity

In all five monkeys, ethanol either reversed or slowed down the abrupt drop in CO_2 combining capacity usually seen in untreated monkeys poisoned with methanol. Chart 1 shows results in a typical pair of experiments

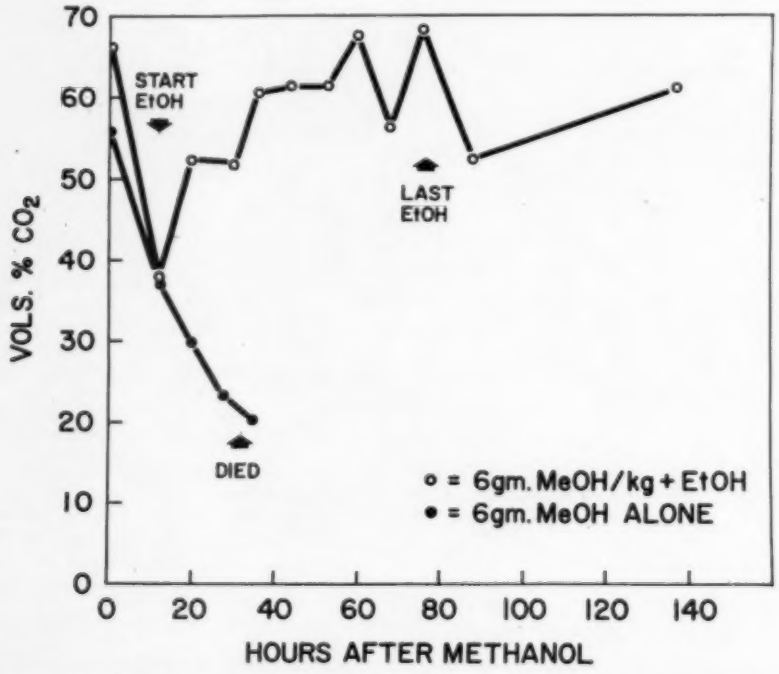

Chart 1 (Gilger, Farkas, and Potts). Plasma CO_2 combining capacity in monkey No. 2.

on the same monkey. In both experiments by 12 hours the CO_2 combining capacity had fallen by over a third of its normal value. When ethanol therapy was started, the CO_2 combining capacity rose to normal levels. In the control experiment the drop in CO_2 combining capacity continued and fell by almost two thirds of normal at death. Table 3 gives our complete data. The first monkey listed showed a pattern similar to the one we have just described. The third monkey, having treatment beginning at 16 hours, was moderately acidotic throughout; by death his CO_2 combining capacity had dropped almost 50 percent. The fourth monkey was a "resistant" one, able to survive the poisoning without treatment. In his case acidosis did not occur under ethanol therapy, but moderate acidosis was present in his untreated control experiment. The last monkey with ethanol beginning at 20 hours had no acidosis until there was a terminal drop in CO_2 combining capacity of about 60 percent. Our series is too small to learn whether there is any relation between length of delay in treatment and degree of acidosis.

Roughly, in comparing percent change from normal plasma CO_2 combining capacities, we found three types of behavior. The untreated group showed a steep downward drop. The group in whom ethanol was started simultaneously with the poisoning[2] showed no drop, and, in fact, mostly increased over the normal. The delayed ethanol group lay in an intermediate position between these other two groups.

TABLE 3

EFFECT OF DELAYED ETHANOL THERAPY ON ACIDOSIS CAUSED BY 6.0 GM. METHANOL/KG. MONKEY AS SHOWN BY PLASMA CO_2 COMBINING CAPACITY

	Hours after MeOH	Vols. % CO_2	
		MeOH and EtOH	MeOH Alone
1. Dionysus	0	29.1	28.4
EtOH 8–68 hr	8	22.5	20.4
	16	27.9	
	17		14.4
	24	19.2	14.3
	28		12.4*
	32	28.5	
	40	17.3	
	48	20.2	
	56	18.9	
	68	24.6	
2. Dalton	0	66.1	55.9
EtOH 12–76 hr	12	37.3	37.2
	20	52.1	29.6
	28		23.2
	29	51.8	
	35		20.0*
	36	60.6	
	44	61.2	
	53	61.4	
	60	67.6	
	68	56.4	
	76	68.3	
	88	52.3	
	137	61.2	
3. Democritus	0	50.1	
EtOH 16 hr to death	16	34.6	
	24	38.8	
	32	24.2	
	41	35.2	
	48	25.9*	
4. Domitian	0	26.8	43.2
EtOH 20–84 hr	20	40.3	31.3
	28	45.8	31.6
	36	40.6	23.6
	44	41.2	
	45		23.2
	52	32.0	
	56		24.9
	60	34.0	
	68	36.6	37.6
	76	37.3	
5. Darwin	0	75.9	
EtOH 20–82 hr	20	60.7	
	28	83.5	
	36	66.6	
	44	72.9	
	52	63.6	
	60	56.4	
	68	54.2	
	76	57.8	
	90	61.6	
	91	28.2*	

* Post-mortem heart puncture.

C. Plasma methanol levels

Plasma methanol levels in monkeys with and without ethanol therapy are shown in Chart 2. The statistical summary is shown in the appendix. The numbers for each line on the chart refer to individual monkeys listed in the table.

Our data indicate that ethanol therapy delays the rate of disappearance of methanol from the blood. This is shown in Chart 2 by

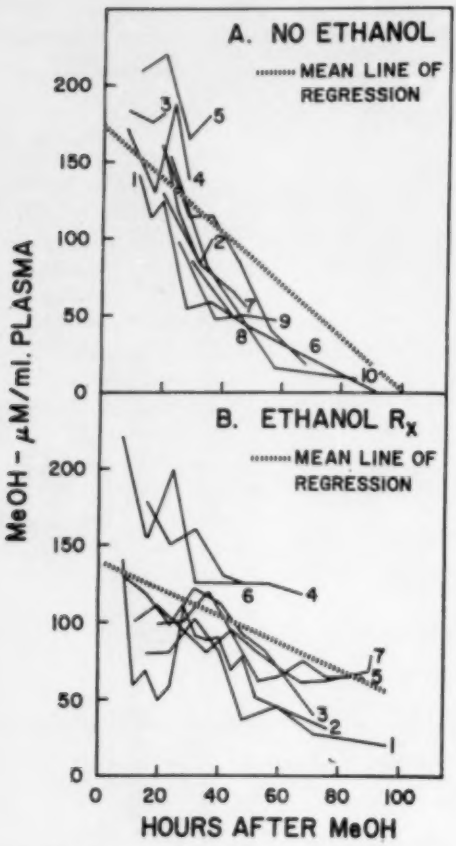

Chart 2 (Gilger, Farkas, and Potts). Plasma methanol concentrations in monkeys given 6.0 gm. MeOH/kg. body weight. (Numerals for each line of this chart refer to individual monkeys listed in Table 4 in the appendix.)

the gradual slope of the mean regression line in ethanol-treated monkeys (B) as compared with the steeper mean regression line in monkeys without ethanol therapy (A). The probability of a difference in slopes this big or bigger, if chance alone caused the differences, is between 0.05 and 0.025.

Details needing amplification are discussed in the appendix.

In spite of the variables mentioned in the appendix—and probably others which are not now obvious to us—we have the desire to try to find order in these chemical determinations from our biologic experiments. This is especially so since we do not consider further ethanol therapy experiments are indicated just now. It appears that ethanol treatment delays the rate of disappearance of methanol from the blood.

Discussion

We have described in a preceding publication how methanol poisoning in primates may be considered a threefold disease.[2] The three components are: I, narcosis; II, metabolic acidosis; III, specific nervous system involvement (retina and basal ganglia). Disease I is due to the toxic effect of the alcohol itself. Diseases II and III are due to a toxic oxidation product of methanol, which is either formaldehyde or a formaldehyde complex. Disease I could conceivably be made worse by ethanol if high enough total alcohol levels were reached. However, this requires a staggering amount of alcohol and is unlikely to be reached in humans. Treatment with base (that is, sodium bicarbonate or sodium lactate) counteracts the acidosis of Disease II, but not the nervous tissue damage of Disease III. We found that ethanol, given early enough, could prevent both Diseases II and III. We also concurred with others that protection by ethanol from Diseases II and III lies in its preempting the enzymic site of methanol oxidation and allowing methanol to be excreted as such or to be oxidized minimally. This concept is supported by our present finding that ethanol slows down the rate of disappearance of methanol from the blood.

Logically, there should be some time limit beyond which enough toxic oxidation product has been formed from methanol so that blindness or death is inevitable, even though additional methanol oxidation is slowed down by giving ethanol. Under the conditions of our experiments this time was 12 to 16 hours. However, even after this time, death was delayed by ethanol therapy. One

would therefore expect that there would be a second, later, period of time during which ethanol could still synergize with other therapeutic agents, notably base, and, in combination, still prevent death. If one were dealing with lower doses of methanol, the safe time would be prolonged; with higher methanol doses, it would be shortened.

There should be an additional set of therapeutic conditions under which ethanol treatment plus base, though delayed, will prevent death from Diseases II and III, but still allow a measure of permanent nervous system damage from Disease III. The data reported do not allow us to speculate on what these conditions might be, but we simply state the desirability of defining them by further experimentation.

It is also desirable to have some knowledge of the time beyond which any ethanol treatment would be futile. Our data do not allow us to determine this directly. However, an absolute limit would be set by the time at which blood methanol values in untreated animals fall to insignificant levels. As one can see in Chart 2, this time lies in the neighborhood of 35 to 48 hours for a single initial dose of 6.0 gm./kg.

On the basis of the above considerations we feel justified in recommending the following ethanol treatment schedule in human methanol poisoning: 0.75 gm./kg. × 1, followed by 0.50 gm./kg. every four hours for about three days. This treatment should be begun as early as possible during the first 48 hours after methanol ingestion. Acidosis, when present, should also be treated with base (that is, sodium bicarbonate or sodium lactate).

Summary

Further observations on ethanol therapy of acute methanol poisoning in the monkey have been presented. Results were reported on the clinical picture, plasma CO_2 combining capacities, and plasma methanol levels. These add support to the conclusion that ethanol successfully competes with methanol for alcohol oxidase, thereby slowing down the oxidation of methanol into a toxic product. Ethanol started more than a half day after large doses of methanol could not in itself save our monkeys' lives. However, this delayed ethanol therapy did prolong the monkeys' lives and offers an important adjunct in the treatment of human methanol poisoning.

Appendix

see Table 4

The chemical determination of plasma methanol is specific. We have methanol levels for 17 normal monkey plasmas. The range is 0 to 27 μM methanol /ml. plasma; the mean is 14 μM/ml.

We have a total of 23 experiments in which monkeys were given 6.0 gm. MeOH/kg. and for which plasma methanol levels were done. In 12 of the 23 experiments the monkeys were treated with ethanol. Results on only seven ethanol-treated experiments are reported. The remaining five were omitted because: clinical courses and deaths in three monkeys were complicated by other diseases (see Materials); in two experiments methanol levels were determined five and 15 months after collection of the samples—although plasmas were stored frozen, we are not sure but that even so they may lose methanol in such a lapse of time. Eleven of the total 23 experiments were ones in which ethanol was not given. Data from one of these 11 experiments were omitted because determinations were made five months after the experiment.

The group of 10 not receiving ethanol therapy was managed in two different ways: six monkeys had no treatment, and the other four received sodium bicarbonate therapy. We believe this combination is justifiable. Four out of our six monkeys having no treatment died too early to afford adequate information on the trend of loss of methanol from the blood. Thus, using results from untreated animals only would give too few monkeys for comparison. That $NaHCO_3$ therapy should be without effect on the plasma methanol level is biochemically sound. Untreated monkeys surviving past 35 hours seem to show the same course as sodium bicarbonate monkeys. (The mean slope for methanol loss from plasma in bicarbonate monkeys is −2.26 against −1.32 for all untreated monkeys. We account for this difference in slope by the early deaths of untreated monkeys before the time at which methanol levels could be expected to drop. For example, the mean slope of untreated monkeys is unduly made less steep by monkey no. 2 who died at less than 19 hours and had a slope of −0.22 and by monkey no. 4 who died at 28 hours and had a slope of −0.51.)

The ethanol group received treatment at different

TABLE 4

COMPARISON OF SLOPES OF LINES OF REGRESSION FOR PLASMA METHANOL CONCENTRATIONS IN MONKEYS GIVEN 6.0 GM. MeOH/KG. BODY WEIGHT

No.	Monkey	EtOH Treatment Starting at: (Hours After MeOH)	Result	B_1 μM MeOH/ml. Plasma/Hour	b_1 $(B_1-\bar{b}_1)$		b_1^2
1	Elagabalus	0	Survival	−0.97	−0.17		0.0289
2	Epandamanas	0	Survival	−0.74		+0.06	0.0036
3	Esau	0	Survival	−0.46		+0.34	0.1156
4	Dionysus	8	Survival	−0.70		+0.10	0.0100
5	J. Dalton	12	Survival	−0.58		+0.22	0.0484
6	Democritus	16	Death	−1.56	−0.76		0.5776
7	C. Darwin	20	Death	−0.59		+0.21	0.0441
			$\Sigma =$	−5.60	−0.93	+0.93	0.8282
			$\bar{b}_1 =$	−0.80			

$$\hat{Y}_1 = 132.51 - 0.80X_1$$

$s_1^2 = \Sigma b_1^2/(n-1) = 0.138$ $\quad s_{\bar{b}_1}^2 = s_1^2/n = 0.0197$

$s_1 = 0.371$ $\quad s_{\bar{b}_1} = 0.140$

No.	Monkey	Treatment	Result	B_2 μM MeOH/ml. Plasma/Hour	b_2 $(B_2-\bar{b}_2)$		b_2^2
1	Elagabalus B.	None	Death	−1.62		+0.08	0.006
2	Epandamanas B.	None	Survival	−2.30	−0.60		0.360
3	Esau B.	None	Death	−0.22		+1.48	2.190
4	Dionysus B.	None	Death	−0.51		+1.19	1.142
5	Dalton B.	None	Death	−1.90	−0.20		0.040
6	Domitian B.	None	Survival	−1.39		+0.31	0.096
7	69 A.	$NaHCO_3$	Survival	−3.23	−1.53		2.341
8	Figaro	$NaHCO_3$	Survival	−2.43	−0.73		0.533
9	Third Base	$NaHCO_3$	Death 9 days	−1.08		+0.62	0.384
10	Home Plate	$NaHCO_3$	Sacrificed 13 days	−2.29	−0.59		0.348
			$\Sigma =$	−16.97	−3.65	+3.68	7.440
			$\bar{b}_2 =$	−1.70			

$$\hat{Y}_2 = 169.76 - 1.70X_2$$

$s_2^2 = 0.826$ $\quad s_{\bar{b}_2}^2 = 0.0826$

$s_2 = 0.909$ $\quad s_{\bar{b}_2} = 0.287$

Slope	B
Mean slope	$\bar{b}$
Deviation from mean slope	b
Summation	Σ
Value of Y estimated from regression	$\hat{Y}$
Sample standard deviation	s
Sample standard deviation of the mean	$s_{\bar{b}}$
Standard error of the difference of the means	$s_{\bar{b}_1-\bar{b}_2}$
"Student's" t	t
Number	n
Degrees of freedom	d.f.
Probability of chance occurrence	P
Numeral subscripts:	
EtOH B	1
No EtOH	2

	n	d.f.	Mean Slope $\bar{b}$	Σb^2
EtOH B	7	6	−0.80	0.828
No EtOH	10	9	−1.70	7.440
		$\Sigma = 15$	$\bar{b}_1 - \bar{b}_2 = +0.90$	$\Sigma = 8.268$

$$s^2 = \frac{\Sigma b_1^2 + \Sigma b_2^2}{\Sigma \text{d.f.}} = 0.551$$

$$s_{\bar{b}_1-\bar{b}_2} = \sqrt{\frac{s^2(n_1+n_2)}{n_1 n_2}} = 0.366$$

$$t = \frac{\bar{b}_1 - \bar{b}_2}{s_{\bar{b}_1-\bar{b}_2}} = 2.459$$

d.f. = 15

$0.05 > P > 0.025$

times and in different amounts. Inspection of Table 4 shows no obvious correlation with slopes; the group is too small to draw conclusions on these matters.

Another detail needing consideration is the range of absolute plasma methanol values for different monkeys at any given time and the possible effect of this range upon the slopes. Normal individual variation is undoubtedly an important factor in absolute values. Unpublished data suggest another factor may be methanol loss during the thawing needed to run analyses of samples so that second determinations on the same plasma are usually low. When carbon dioxide combining capacities were done before methanol levels, technical difficulties and larger numbers of samples could also increase

the period of thawing. Care was taken to make thawing periods of all samples from any one experiment the same; thus, although thawing probably accounts for differences in absolute values, it should not have changed our slopes. Vomiting caused negligible loss of the original dose and had no measurable effect on our methanol levels. Our data show no influence of body weight or estimated maturity of the monkey on either range or slope.

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PATHOLOGY OF CORNEA GUTTATA*

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Vogt[1] first described the clinical finding of cornea guttata as seen in the early stage of endothelial and epithelial dystrophy of Fuchs.[2] Vogt found also that the slit-lamp finding of the "droplike endothelial prominences" of the guttate corneal dystrophy is caused by many hyaline excrescences on the posterior surface of Descemet's membrane. Later studies confirmed this finding and furthermore revealed that the development of the hyline excrescences goes along with a progressive primary degeneration of the corneal endothelium[3-12] (figs. 1 and 2). The same studies showed that these excrescences are composed of the same substance as Descemet's membrane[4] (fig. 3) and it has become very probable that they are a secretion product of abiotrophic endothelial cells (Verhoeff[5]). Thinning and perforation of the endothelium were found on the larger excrescences[4,9] (fig. 2). This loss of endothelium on top of the hyaline excrescences may explain the abnormal hydration, secondary scarring, and bullous epithelial changes found in the late stages of Fuchs corneal dystrophy.

As yet cornea guttata has only been described as a primary degenerative disease of unknown etiology representing one early stage of Fuchs corneal dystrophy. It is known to occur as a familial dystrophy.[13] The single lesions of the cornea guttata are very similar to the warts or a Hassal-Henle bodies of the peripheral corneal endothelium which are considered the product of primary senile degeneration. The present paper, however, contains the description of cases in

* From the Department of Ophthalmic Surgery and the Laboratory of Neuropathology of the University of Michigan Medical Center. Presented before the East-Central Section, January 5, 1959, Cincinnati, Ohio.

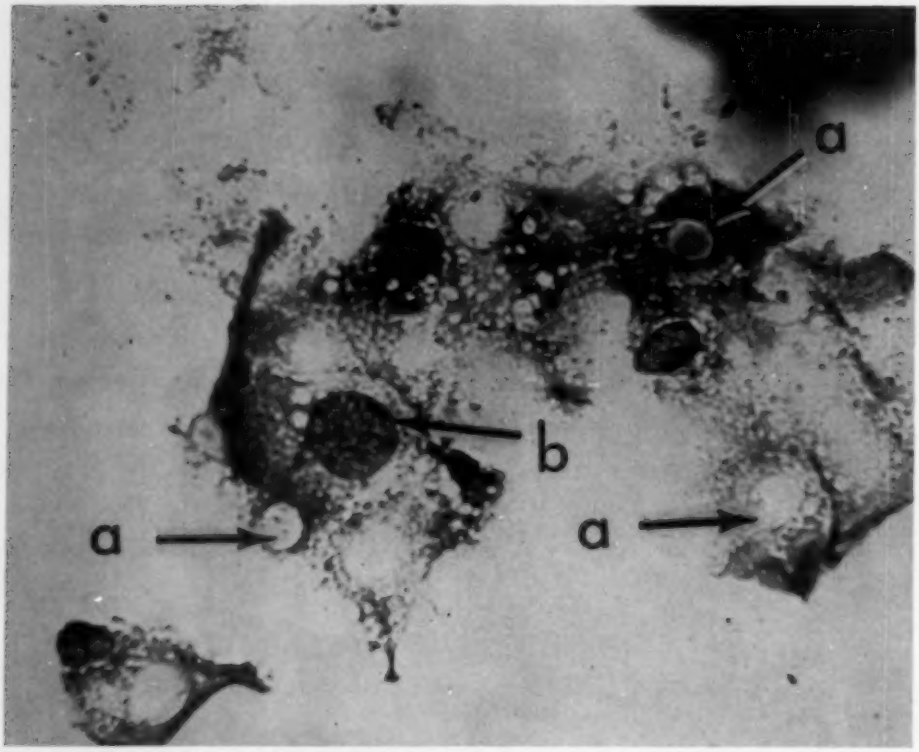

Fig. 1 (Wolter and Larson). Early stages of hyaline excrescences in the protoplasm of endothelial cells (a) of the central cornea of the 56-year-old white man with Fuchs corneal dystrophy. (b) Endothelial nuclei. (Flat section through the endothelium, panoptic technique of del Rio Hortega, photomicrograph.)

which a peculiar type of cornea guttata with bizarre geographic arrangement of the hyaline excerescences occurred secondary to old deep keratitis.

Case reports

Case 1

This 70-year-old white woman was first seen in this eye clinic on June 5, 1958. She gave a history of an eye infection, O.U., at the age of 12 years which was associated with influenza. This resulted in dense corneal scars and poor vision, O.U. Optical iridectomies, O.U., were done several months later which had improved her eye sight. However, at the time of the examination she had noted a progressive loss of vision for many years.

The eye examination on June 5, 1958, revealed her visual acuity to be counting fingers at one foot in the right eye and counting fingers at three feet in the left eye. Other findings of interest were: slit-lamp examination showed both corneas to have marked band keratopathy with scarring and calcifications. There was some peripheral vascularization in the superficial layers of both corneas. No deep vessels were seen. The anterior chamber, O.U., appeared of about normal depth. There were optical iridectomies inferiorly in both eyes. The lenses were not well seen in both eyes but appeared cataractous. Funduscopic examination was not possible. However, the patient had color perception and light projection, O.U. Tonometer tension was 23.8 mm. Hg in the right eye and 21.9 mm. Hg in the left eye (corrected Schiøtz). The intraocular pressure, O.U., appeared also normal at finger palpation. Chest X-ray films, serologic examination, and hematocrit determination were all normal.

The diagnosis of a band keratopathy following old keratitis, O.U., was made and a penetrating keratoplasty was done on the right eye on August 4, 1958. The postoperative course was without complications. The graft remained clear until last seen at time of discharge on August 12, 1958. However, a senile cataract of the nuclear type was found.

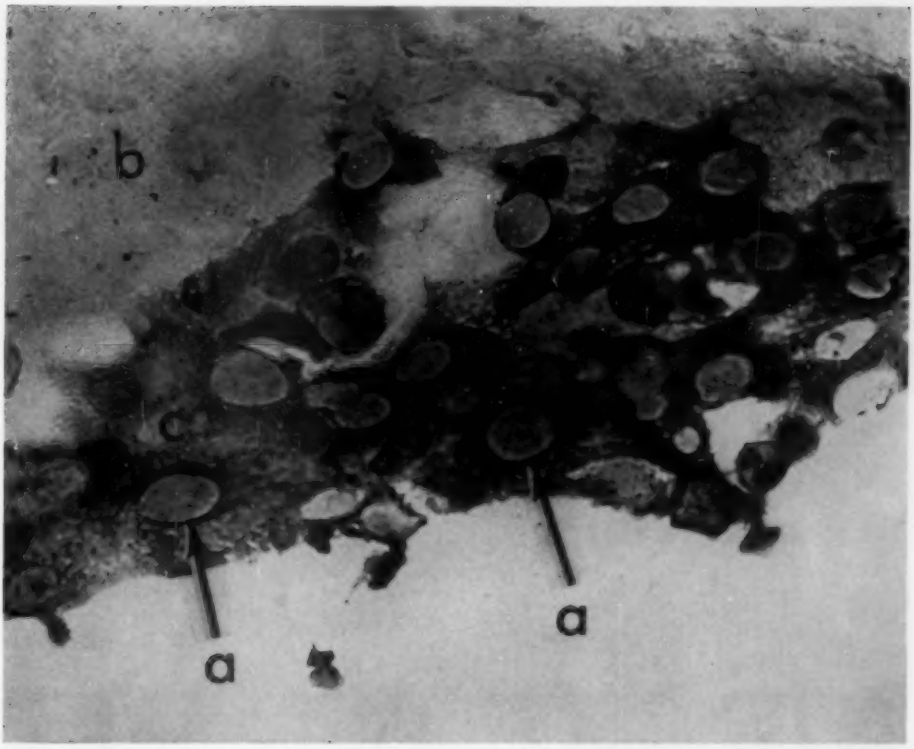

Fig. 2 (Wolter and Larson). Large hyaline excrescences (a) in the endothelium of the central cornea of a 56-year-old white man with Fuchs corneal dystrophy. (Transitional section through Descemet's membrane (b) and the endothelium (c), panoptic technique of del Rio Hortega, photomicrograph.)

The removed central corneal button of the right eye of this patient was immediately fixed in ammonium bromide formalin (Cajal solution). This button measured 6.5 mm. in diameter. It was cut in flat sections on the freezing microtome. The panoptic technique of the silver carbonate methods of del Rio Hortega[9] was used to stain the sections.

The corneal epithelium was found to be irregular in thickness and arrangement, but it was continuous. There was extensive old scarification of the stroma with some calcification in the superficial layers. No blood vessels were found in the corneal button. Descemet's membrane was of about normal thickness. However, numerous hyaline excrescences were found to cover the posterior surface of Descemet's membrane. In some areas only few of these round excrescences were seen (fig. 4). Other areas showed rather dense accumulations of these small isolated hyaline bodies (fig. 5). There were also regions with hyaline bodies of the same kind that had become confluent and formed irregular lines (figs. 6 and 7). They were intermixed with single hyaline excrecences (fig. 7) and also formed bizarre geographic patterns (fig. 8). The endothelium on top of these hyaline bodies showed advanced local thinning and was also somewhat atropic as a whole.

This case of extensive band keratopathy following old keratitis showed histologically the typical endothelial changes of cornea guttata. However, the arrangement of most of the hyaline excrescences (guttae) was in lines or bizarre geographic patterns. This has to our knowledge never been described in cases of Fuchs corneal dystrophy. It is our impression that the cornea guttata in this case must have been of a secondary nature and a sequela of the old keratitis.

In some other cases—all of luetic interstitial keratitis in females—similar changes as in the first case were observed clinically. These cases will be briefly described in the following:

CASE 2

This 37-year-old white woman was first seen in this clinic on August 22, 1955, and has been followed and treated here since that time. Her vision was

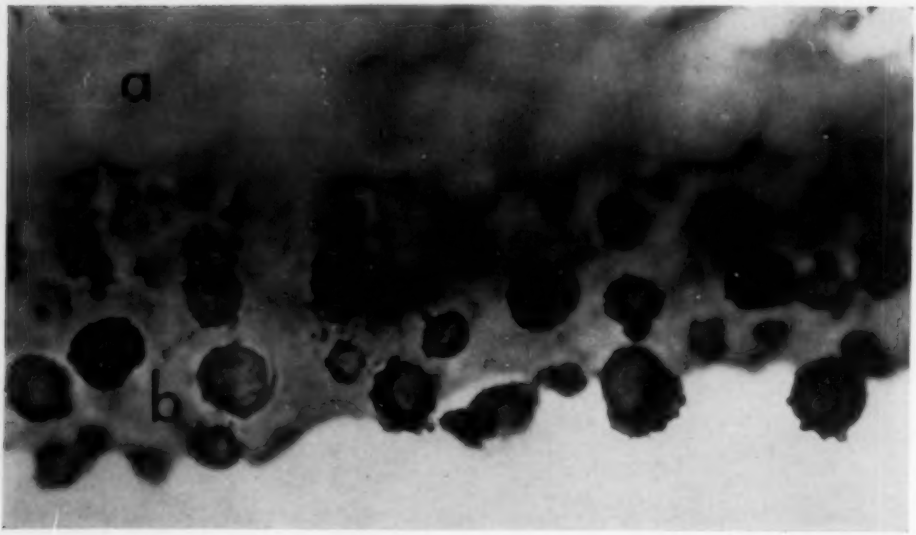

Fig. 3 (Wolter and Larson). Isolated hyaline excrescences on the posterior surface of Descemet's membrane in a case of Fuchs corneal dystrophy. The endothelium is lost in this section. (Transitional section through Descemet's membrane (a) and the layer of the hyaline excrescences (b), panoptic technique of des Rio Hortega, photomicrograph.)

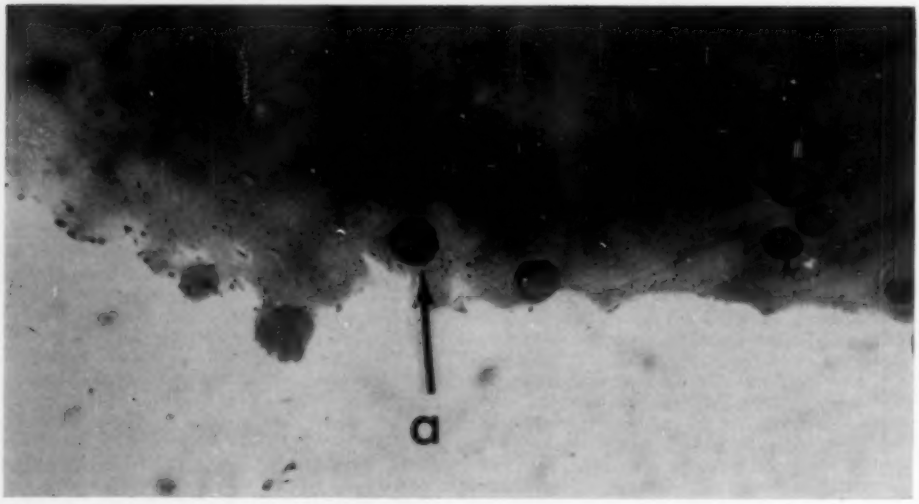

Fig. 4 (Wolter and Larson). An area with a few isolated guttae on the posterior surface of Descemet's membrane of the first case with secondary cornea guttata in old keratitis. (a) Hyaline excrescences. (b) Descemet's membrane. (Transitional section through Descemet's membrane, panoptic technique of del Rio Hortega, photomicrograph.)

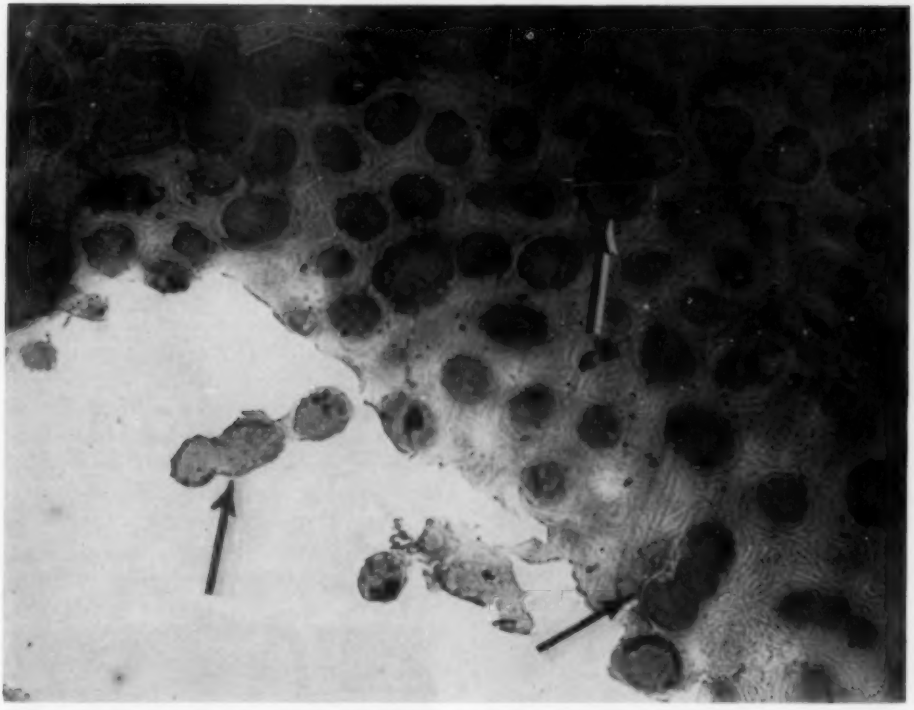

Fig. 5 (Wolter and Larson). An area with densely arranged guttae on Descemet's membrane of the first case with secondary cornea guttata in old keratitis. Some of the hyaline excrescences are confluent (arrows). (Flat section, panoptic technique of del Rio Hortega, photomicrograph.)

found and has remained 20/30 in the right eye and hand motion at five feet in the left eye. Our diagnosis was: old luetic interstitial keratitis, O.U., old luetic chorioretinitis, O.U., secondary glaucoma, O.U., secondary optic nerve atrophy, O.S., and glaucomatous cupping, O.D. Her right visual field showed extensive glaucomatous changes. The gonioscopy revealed some peripheral anterior synechias and blood vessels in the angle, O.U. The patient has been on a conservatice program with pilocarpine, eserine, and Diamox since she was considered a poor surgical risk.

The corneas of this patient showed extensive opacification and diffuse haze of the stroma with deep vascularization (ghost vessels). This was more extensive in her right than in the left eye. Both corneas showed a peculiar type of central cornea guttata which was also more marked in the right eye. Many small isolated guttae were seen, but the outstanding findings were lines and geographic patterns which were obviously composed of rows of hyaline excrescences. Figure 9 shows a drawing of the arrangement of the rows of hyaline bodies as seen with the slitlamp in the right eye of this patient.

The isolated hyaline bodies as well as the rows of the same bodies were clearly visible in this case. We have no doubt that they represented a special type of cornea guttata just as that seen histologically in our first case.

Case 3

This 51-year-old white woman was referred on November 3, 1958, to our clinic from the Neurology Department of this University Medical Center. Her vision was found to be 20/40 in the right eye and 20/20 in the left. She showed an "incomplete" Argyll-Robertson pupil, O.U., and an old interstitial keratitis, O.U. Blood serology was negative. A lumbar puncture done on November 7, 1958, was completely normal. A treponema pallidum immobilization test, however, was positive in the blood but negative in the spinal fluid.

At slitlamp examination both corneas of this patient showed some opacities and general haze of the stroma with many deep ghost-vessels and blood-filled vessels, O.U. There was again this peculiar cornea guttata with lines and geographic patterns on

Fig. 6 (Wolter and Larson). A hyaline ridge composed of many hyaline excrescences on Descemet's membrane (a) of the first case with secondary cornea guttata in old keratitis. (Transitional section through Descemet's membrane, panoptic technique of del Rio Hortega, photomicrograph.)

the posterior surface of Descemet's membrane of both eyes. Figure 10 gives an impression of the arrangement of the guttae in the left eye of this patient as seen with the slitlamp. The changes in the right eye were very similar and the changes in both eyes were very much like those observed in our second case.

CASE 4

This is the case of a 46-year-old white woman seen at the eye clinic of the Wayne County General Hospital in Eloise, Michigan. She was deaf and virtually blind. The following diagnoses were listed: congenital lues with nerve deafness, blindness, O.U., pendulant nystagmus, O.U., divergent strabismus, O.D., and old interstitial keratitis, O.U. Her vision was found to be amaurosis in her right eye and light projection and color perception in the left eye. The slitlamp examination revealed extensive nebular scarring of the corneal stroma and many deep blood vessels, O.U. Descemet's membrane and the endothelium were covered with much pigment. In the left eye the posterior corneal surface appeared otherwise normal. In the right eye, however, a very distinct branching formation of hyalinelike substance was seen paracentrally on Descemet's membrane. Figure 11 gives an impression of the shape, size, and arrangement of this formation. With the highest magnification it could again be seen that the prominent ridge of this formation was composed of many round globules (guttae). No isolated hyaline bodies were seen besides this formation.

DISCUSSION

From the literature we know that hyaline ridges or networks may remain attached to the endothelial surface of the cornea as sequelae of syphilitic interstitial keratitis[15] and other forms of deep keratitis.[16] Such formations are considered hyalinized remnants of fibrinous exudates of the anterior chamber or of keratitic precipitates. The observation of a special type of cornea guttata secondary to keratitis seems to be new.

The hyaline excrescences which were seen

Fig. 7 (Wolter and Larson). Long hyaline ridges composed of many hyaline excrescences on Descemet's membrane of our first case of secondary cornea guttata in old keratitis. Some isolated hyaline bodies are seen between the ridges. (Transitional section through Descemet's membrane, panoptic technique of del Rio Hortega, photomicrograph.)

histologically and clinically on Descemet's membrane of the cases here demonstrated had all the characteristics of those seen in the primary corneal dystrophy of Fuchs. The only difference was that in these cases of secondary cornea guttata most of the hyaline excrescences were confluent with each other and arranged in lines or peculiar geographic patterns. We believe that the hyaline excrescences of the cornea guttata in Fuchs corneal dystrophy are a pathologic secretion product of degenerating endothelial cells. In these cases of secondary cornea guttata the degeneration of the endothelial cells probably is secondary to the old deep keratitis which was found in all our cases. We cannot as yet explain why this degeneration involves endothelial cells in such peculiar patterns. It seems possible that deep blood vessels or secondary clearing lines may somehow induce the formation of the rows of guttae. It must be emphasized that it is one of the characteristics of Fuchs corneal dystrophy that the hyaline excrescences are isolated and usually do not become confluent. Fuchs corneal dystrophy is three times as common in females as in males.[4] In this respect it may be noted that all our cases of secondary cornea guttata were seen in females. The fact that in our

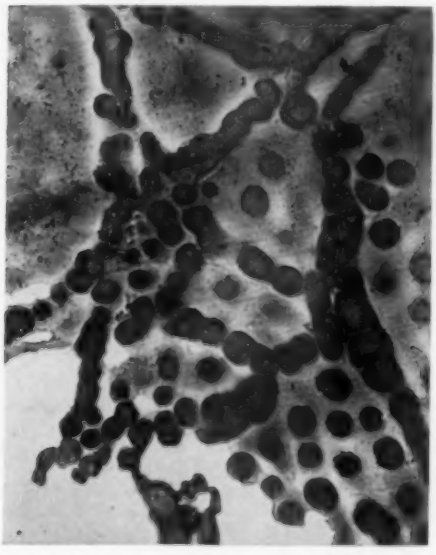

Fig. 8 (Wolter and Larson). Geographic pattern composed of many confluent hyaline excrescences on Descemet's membrane of our first case of secondary cornea guttata in old keratitis. There are also many isolated guttae. (Transitional section of Descemet's membrane, panoptic technique of del Rio Hortega, photomicrograph.)

Fig. 9 (Wolter and Larson). Drawing reconstruction of the lines and patterns of the secondary cornea guttata of the right eye of the second case as seen with the slitlamp.

Fig. 10 (Wolter and Larson). Drawing reconstruction of the lines and patterns of the secondary cornea guttata of the left eye of the third case as seen with the slitlamp.

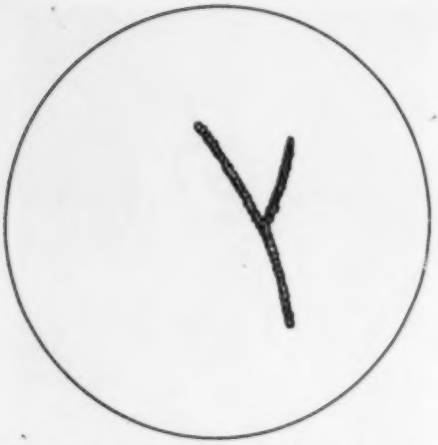

Fig. 11 (Wolter and Larson). Drawing reconstruction of the lines and the shape of the single formation of secondary cornea guttata in the right eye of the fourth case as seen with the slitlamp.

Case 4 this secondary cornea guttata was observed unilaterally is of some importance since this further indicates the secondary nature of this finding. Primary cornea guttata of Fuchs dystrophy is usually bilateral.

The present paper has its shortcomings. The first case which allowed for a histologic demonstration of the cornea guttata showed such a dense scarring of the stroma that the guttae could not be seen clinically. And in the other cases where the secondary cornea guttata was seen clinically the cornea could not be examined histologically. However, after studying many cases of primary cornea guttata of the Fuchs type clinically as well as histologically, we are quite certain that the case that was studied histologically and the cases which were seen only clinically are of the same entity. We would like to call the finding of this peculiar geographic guttate corneal dystrophy "secondary cornea guttata."

It is possible that all the cases of this paper were cases of luetic interstitial keratitis. In two cases, however, we cannot be sure of that diagnosis. All we want to say at this time is that all these cases with secondary cornea guttata exhibited an old keratitis which involved the deep corneal stroma. We hope that further studies may reveal additional facts about the nature and occurrence of secondary cornea guttata.

Summary

The finding of a secondary type of cornea guttata is described histologically and clinically. The hyaline excrescences in secondary cornea guttata are arranged in lines and geographic figures. Secondary cornea guttata was found in cases of old deep keratitis. Primary cornea guttata in Fuchs dystrophy is discussed for comparison.

University Medical Center.

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THE EFFECT OF SYMPATHOMIMETIC DRUGS UPON THE AMPLITUDE OF ACCOMMODATION*

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In 1937 Cogan[1] presented a comprehensive analysis of the importance of the sympathetic nervous system in the accommodative process. This stimulated a number of physiologic experiments upon a variety of mammalian preparations. The present experiments were designed to expand upon Cogan's original observations of the effects of sympathomimetic drugs upon the accommodative process of the human eye.

METHOD

Two different procedures were employed to study the changes in accommodation brought on by these drugs. The first of these employed a stigmatoscope which presented a single spot of light viewed on an ophthalmic letter chart (84.5 ft.-L). The spot was adjusted to sharpest focus by a procedure outlined in detail elsewhere,[2] and in this way the conjugate focus to the retina of the accommodating eye was ascertained. By placing lenses before the eye and requiring the observer to resolve the details of the letter chart while adjusting the stigmatoscope to its sharpest focus, it is possible to measure the accommodative response to a variety of accommodative stimuli over a wide range. In this way the far and near points of accommodation could be ascertained as well as the effectiveness of a given lens in evoking a response. All measurements were made while viewing through a 2.0-mm. artificial pupil.

The second procedure employed the subjective Badal optometer in order to measure the near point of accommodation. This instrument consists of a calibrated optical bench with a 2.0 mm. artificial pupil placed at the anterior focal point of a strong (8.0D.) plus lens. A minus lens (5.5D.) is placed immediately in front of the artificial pupil to keep the test target within a convenient range. Standard overhead lighting was used to illuminate the target. This was a series of horizontal black and white bar grating (each with narrower separation than the one above it). The chart luminance was 4.80 ft.-L. The system has the advantage that changes in position of the target have no effect at all upon either its brightness or its apparent size.

Topically administered drugs were instilled every two minutes for a total of eight drops. The lacrimal sac was occluded with the finger. Subconjunctival injection was performed after topical 0.5-percent tetracaine anesthesia. Drugs administered were

1. Ten-percent phenylephrine hydrochloride solution (neosynephrine)
2. Two-percent epinephrine bitartrate (Epitrate)
3. Four-percent epinephrine bitartrate[3]

* From the Departments of Ophthalmology and Pharmacology, University of Michigan. This investigation was supported in part by U.S.P.H.S. Grant No. 2B-5163. Presented before the East-Central Section, Cincinnati, Ohio, January 5, 1959.

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‡ Senior research fellow, National Institutes of Health.

4. One/one thousand epinephrine hydrochloride (for injection)
5. One-percent cyclopentolate hydrochloride (Cyclogyl)
6. Five-percent homatropine hydrobromide
7. Two-percent lidocaine hydrochloride (Xylocaine)

Results and Comment

In the first subject (M. A.) five-percent homatropine was instilled intensively until the eye was no longer able to accommodate (fig. 1). The recession of the far-point was one diopter more than could be obtained by stimulating the eye with plus lenses alone. When the eye was injected subconjunctivally with 0.2 cc. of 1/1,000 epinephrine hydrochloride, there was a marked decrease in the amplitude of accommodation. The effect was most marked at the near-point and amounted to a 2.5D. loss of accommodation on both the optometer and stigmatoscope (figs. 1 and 3). At the far-point the change was less dramatic, amounting to only 0.5D. to 0.75D.

An injection of 0.15 cc. of 1/1,000 epinephrine hydrochloride in a second subject (R. D. B.) caused a 2.5D. loss of accommodative amplitude with all the change occurring at the near-point. Again the maximum effect was 30 minutes after injection (fig. 3).*

In each subject the slope of the stimulus-response curve (within limits of the ability of the eye to respond) was essentially unchanged by the epinephrine.

When the 0.2 cc. of 1/1,000 epinephrine was combined with an equal volume of two-percent lidocaine,† a 5.0D. to 6.0D. loss of accommodation was noted (fig. 2). The onset was apparent in 10 minutes and the marked effect lasted a full hour (fig. 3). In spite of the marked change at the near-point the latent hyperopia never exceeded that which could be revealed by repeated instillations of one-percent cyclopentolate (fig. 2).

In Figures 1 and 2 the point of interest with regard to the far-point is that strong sympathomimetic stimulation of the eye does not cause recession of the far-point as much as that caused by parasympathetic blocking alone (with homatropine or Cyclogyl). These findings are similar to those of Hartgraves and Kronfeld[8] who found that subconjunctival epinephrine did not increase the refraction over the manifest findings, but that topical atropine did.

Currently, there have been suggestions by Siebeck,[9] Morgan,[10] and others that accommodation for distances greater than 0.75D. is negative while that for distances nearer than 0.75D. is positive. It is also postulated that negative accommodation is mediated via the sympathetic nervous system and positive accommodation is mediated via the parasympathetic nervous system. This value of 0.75D. has been related by some authors to the de-

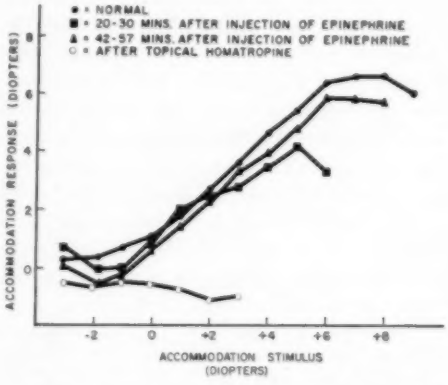

Fig. 1 (Biggs, Alpern, and Bennett). The effects of topical homatropine and the subconjunctival injection of epinephrine upon the stigmatoscopic determinations of the accommodation response evoked by various lenses before the right eye (M. A.)

* Since the effects were so transient, it was not possible to study the accommodative-convergence relationships as reported by Christoferson and Ogle,[4] Sabin and Ogle,[5] and Alpern.[6]

† Pharmacologic properties of lidocaine which are of importance in its apparent enhancement of the epinephrine effect include:[7] 1. Local anesthetic action (blocking of nerve conduction and depression of the muscle fiber itself); 2. atropine like qualities; 3. antihistaminic activity.

gree of myopia in space and nocturnal myopia. However, the data presented here would also emphasize that relaxation of the parasympathetic tonus is essential in obtaining this so-called negative accommodation.

Whether one chooses to explain the effect of the sympathetic nervous system upon the ciliary body as a result of vascular change (Fleming[11]) or as a result of direct stimulation of a portion of the musculature (Cogan[1]), the reason that the effect is so much greater at the near-point than at the far-point must be explained. In either case the explanation may lie in the fact that at the far-point the mechanical limit of the system is approached. The lens may be unable to flatten beyond the state in which it is found in "complete cycloplegia."

Figure 3 demonstrates the changes at the near-point of accommodation recorded on the subjective optometer for various times following injection. The effects were prompt, but transient, having returned to normal by two hours. No effect was noted in the non-injected eye. The changes recorded on the optometer correlated well with those in the stigmatoscope.

Unfortunately, subconjunctival injections

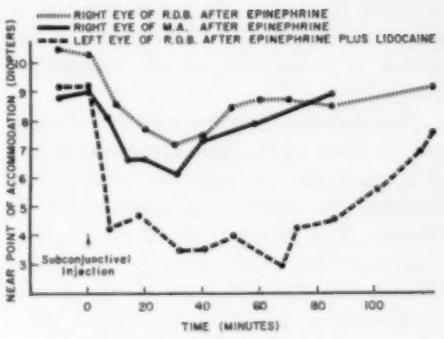
RIGHT EYE OF R.D.B. AFTER EPINEPHRINE
RIGHT EYE OF M.A. AFTER EPINEPHRINE
LEFT EYE OF R.D.B. AFTER EPINEPHRINE PLUS LIDOCAINE
NEAR POINT OF ACCOMMODATION (DIOPTERS)
Subconjunctival injection
TIME (MINUTES)

Fig. 3 (Biggs, Alpern, and Bennett). Change in the near-point of accommodation as measured by the subjective optometer at various time intervals following the injection of epinephrine.

left the eye markedly infected and caused low grade iritis in each instance. Therefore, further attempts to induce changes in accommodation were made only with topical administration of the drug. Intensive administration of a 10-percent solution of phenylephrine hydrochloride—one drop every two minutes for a total of eight drops—caused a recession of the near-point averaging 0.66D. in five subjects. The effect was noted in each case 30 minutes after instillation began and had disappeared in two hours (fig. 4). Mydriasis was marked by 30 to 40 minutes but lasted long after accommodation

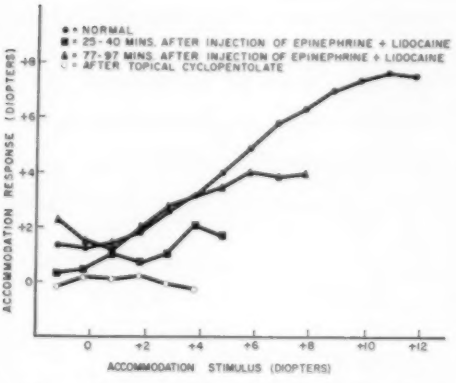
● = NORMAL
■ = 25-40 MINS. AFTER INJECTION OF EPINEPHRINE + LIDOCAINE
▲ = 77-97 MINS. AFTER INJECTION OF EPINEPHRINE + LIDOCAINE
○ = AFTER TOPICAL CYCLOPENTOLATE
ACCOMMODATION RESPONSE (DIOPTERS)
ACCOMMODATION STIMULUS (DIOPTERS)

Fig. 2 (Biggs, Alpern, and Bennett). The effects of topical cyclopentolate and subconjunctival injection of epinephrine plus lidocaine on the stigmatoscopic determinations of the accommodation response evoked by various lenses before the left eye (R. D. B.)

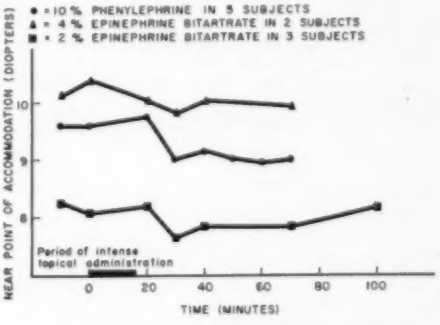
● = 10 % PHENYLEPHRINE IN 5 SUBJECTS
▲ = 4 % EPINEPHRINE BITARTRATE IN 2 SUBJECTS
■ = 2 % EPINEPHRINE BITARTRATE IN 3 SUBJECTS
NEAR POINT OF ACCOMMODATION (DIOPTERS)
Period of intense topical administration
TIME (MINUTES)

Fig. 4 (Biggs, Alpern, and Bennett). The effect of topical application of certain sympathomimetic agents on the near-point of accommodation as measured by the subjective optometer at various time intervals following drug administration.

returned to normal. When the drops were administered once every 15 minutes, it was difficult to demonstrate any effect on accommodation.

Phenylephrine contains only the vasoconstrictor phase of the sympathomimetic group of drugs. Epinephrine contains both vasoconstricting and vasodilating potential but its pressor effect is many times greater than phenylephrine. A two-percent epinephrine bitartrate solution, used intensively as with phenylephrine, produced an average recession of the near point of 0.5D. in three subjects (fig. 4). A mydriasis of approximately two mm. was produced.

When the concentration of epinephrine was increased to four percent, the stinging sensation was so marked that the lacrimation was profuse. Accommodative loss was insignificant.

Summary

Subconjunctival injection of epinephrine was shown to cause a marked loss of accommodative amplitude with virtually all the change occurring at the near-point. This effect was prompt, but quite transient. This sympathomimetic stimulation could not cause a recession of the far-point as much as that caused by parasympathetic blocking alone.

The loss in accommodative amplitude was effected without change in the slope to the stimulus-response curve within limits of the ability to respond.

Intensive topical administration of phenylephrine hydrochloride and epinephrine bitartrate caused only a slight recession of the near-point. No effect was noted at dosage levels which the clinician might employ for refraction purposes.

1313 East Ann Street.

References

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SECTION MEETINGS

of the

ASSOCIATION FOR RESEARCH IN OPHTHALMOLOGY, INC.

Auditor's Report

Officers
Directory of Members
Geographical List
Contributions to Friedenwald Fund

PUBLICATIONS COMMITTEE

FRANK W. NEWELL
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LORAND V. JOHNSON

Southern Section ..... Dallas, Texas, November 3, 1958
A. E. Meisenbach, Jr., *Chairman*

Western Section ..... San Francisco, California, November, 1958
Frank C. Winter, *Chairman*

Eastern Section ..... New York, New York, November, 1958
Jerry H. Jacobson, *Chairman*

Southeastern Section ..... Winston-Salem, North Carolina, December, 1958
Richard G. Weaver, *Chairman*

Midsouthern Section ..... No Meeting, 1958
Samuel B. Johnson, *Section Secretary*

East Central Section ..... Cincinnati, Ohio, January, 1959
Donald J. Lyle, *Chairman*

Mid-Winter National Meeting ..... Edgewater Park, Mississippi, February, 1959
James H. Allen, *Chairman*

Midwestern Section ..... Denver, Colorado, April, 1959
George S. Tyner, *Chairman*

National Meeting ..... Atlantic City, June, 1959
Lorand V. Johnson, *Secretary-Treasurer*

AUDITOR'S REPORT

Association for Research in Ophthalmology, Inc.

January 19, 1959

To the Members of the Board of Trustees
Association for Research in Ophthalmology, Inc.
Cleveland, Ohio

Gentlemen:

I have examined the cash basis accounting records for the Association for Research in Ophthalmology, Inc., as of December 31, 1958, and the related transactions of receipts and disbursements for the year then ended. The examination was made in accordance with generally accepted auditing standards and included such tests of the accounting records and such other procedures as I considered necessary in the circumstances.

Despite the fact that no confirmation of dues in arrears was made directly with the members, in my opinion the accompanying statements present fairly the fund balances of the Association for Research in Ophthalmology, Inc., as of December 31, 1958, and the receipts and disbursements for the year then ended, in conformity with generally accepted cash basis accounting principles and applied on a basis consistent with that of the prior year.

Very truly yours,
(Signed) MARTIN C. BLAKE.

February 4, 1959

Board of Trustees
Association for Research in Ophthalmology, Inc.
Cleveland, Ohio

Gentlemen:

I have reviewed the treasurer's report of the financial status of the Association for Research in Ophthalmology, Inc., for the year 1958, together with the auditor's report and the supporting documents.

These appear to be in order and are hereby approved by me as a member of the auditing committee.

Very truly yours,
(Signed) HENRY F. ALLEN.

February 2, 1959

Board of Trustees
Association for Research in Ophthalmology, Inc.
Cleveland, Ohio

Gentlemen:

After reviewing the treasurer's report together with the report of the auditor for the fiscal year ending December 31, 1958, I find the financial status of the organization apparently as has been indicated.

I therefore approve this report as a member of the auditing committee.

Very truly yours,
(Signed) WEBB P. CHAMBERLAIN, JR., M.D.

Balance on December 31, 1957		$10,451.19
Add—Receipts:		
Dues Collected	$10,730.00	
Union Electric Co. Dividends	114.00	
Supplements	18.00	
Banquet Proceeds	675.00	
Credit from Foreign Exchange	.55	
Total Receipts		$11,537.55
		$21,988.74
Deduct—Disbursements:		
Convention Expenses:		
Dinners	$ 968.76	
Programs, mailing, notices, etc.	892.26	
Expense of Secretary-Treasurer	250.00	$ 2,111.02
Publication—June Supplement[1]		1,000.00
Stationery, supplies, printing		932.17
Postage—Shipping		171.48
Rent—Office Space		600.44
Auditing		115.00
Telephone		160.83
Insurance—Surety Bond—Secretary-Treasurer		12.50
Board of Trustees' Meeting—May, June, October		116.40
Contribution		84.36
Friedenwald Lectureship—Cash Award		500.00
Friedenwald Plaques for 1957 and 1958		182.36
Office Equipment		727.65
Miscellaneous: supplements, rosters, etc.		159.33
Salary Gross	$3,039.96	
Less—Withholding and FICA tax withheld	516.75[2]	2,523.21
Withholding tax and FICA[3]		516.75
Employer's portion of FICA[3]		115.41
International Council—Dues		140.43
Proctor Medals for 1959-1960		339.00
Total Disbursements		$10,508.34
Balance December 31, 1958		$11,480.40

[1] Payment for October and November issues unpaid at December 31, 1958.
[2] Includes payment of FICA for 1957, and income tax withheld in 1957.
[3] Includes employer's portion of FICA for 1957.

SUMMARY OF MEMBERSHIP BY YEARS THROUGH DECEMBER 31, 1958

Years Ended December 31	Total Members		
1958	1,190[1]	1944	283
1957	979	1943	—[4]
1956	930[2]	1942	281
1955	822[2]	1941	279
1954	815[2]	1940	270
1953	713[2]	1939	268
1952	672[3]	1938	272
1951	556[3]	1937	249
1950	509[3]	1936	240
1949	474	1935	245
1948	422	1934	230
1947	306	1933	219
1946	324	1932	203
1945	—[4]	1931	193
		1930	134

[1] Includes provisional members for 1959.
[2] Official membership whether dues paid or not.
[3] Includes only paid-up members.
[4] Statistical information not available.

Statement of Dues Collected for Year Ended 12-31-58

Dues collected for prior years		
1954—1 active member @ $5.00	$ 5.00	
1954—1 educational member @ $2.00	2.00	
1955—1 active member @ $5.00	5.00	
1955—1 educational member @ $2.00	2.00	
1956—2 active members @ $10.00	20.00	
1956—2 educational members @ $3.00	6.00	
1956—1 educational member @ $2.00	2.00	
1957—9 active members @ $10.00	90.00	
1957—3 educational members @ $3.00	9.00	$ 141.00
Dues collected for current year		
1958—889 active members @ $10.00	8,890.00	
95 educational members @ $3.00	285.00	
39 sustaining members @ $25.00	975.00	
1 sustaining member @ $35.00	35.00	
1 sustaining member @ $50.00	50.00	10,235.00
Dues collected for subsequent years		
1959—2 active members @ $10.00	20.00	
1959—2 educational members @ $3.00	6.00	
1960—1 active member @ $10.00	10.00	36.00
Dues collected from new members approved		
1959—9 active members @ $10.00	90.00	
1959—4 educational members @ $3.00	12.00	
1959—2 sustaining members @ $25.00	50.00	152.00
Dues collected from provisional members		
1959—11 active members @ $10.00	110.00	
1959— 2 educational members @ $3.00	6.00	
1959— 2 sustaining members @ $25.00	50.00	166.00
Total dues collected in year 1958		$10,730.00

Cash and Securities in Funds

	General Fund	Proctor Medal Fund	Friedenwald Memorial Fund	Total
Cash 12-31-57	$10,451.19	—0—	—0—	$10,451.19
Cash 12-31-58	11,480.40	—0—	$ 7,323.77[1]	18,804.17
Increase	$ 1,029.21		$ 7,323.77	$ 8,352.98
Securities		$10,184.63[2]	$ 2,053.13[3]	$12,237.76
Total Cash & Securities on 12-31-58				$31,041.93

[1] On deposit in the Cambridge Savings Bank and in custody of Dr. David G. Cogan, Chairman of the Friedenwald Memorial Committee.

[2] U. S. Treasury Bonds paying 2½% due 1967-72. At December 31, 1958, coupons amounting to $250.00 matured in 1958, were affixed to the bonds.

[3] 75 Shares of Union Electric Co. @ 27⅜, initial value of gift.

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1959

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Weymouth, Frank W.
Wilson, Warren A.

Menlo Park
Stevenson, Thomas C.

Monterey
True, William R.

North Hollywood
Fields, Jack
Nielsen, Ray H.

Oakland
Bell, Dudley P.
Garron, Levon K.
Gump, Millard E.
Howell, Homer P.
Moorman, Lemuel T.
Schnoor, Thomas G.

Oceanside
Mannis, Aaron A.

Ojai
Hirsch, Monroe J.

Palm Springs
Grow, Kenneth A.
Piser, Alfred L.

Palo Alto
Bock, Rudolf H.
Flocks, Milton
Poore, John C.
Tanner, Owen R.
Winter, Frank C.
Zweng, Harold C.

Pasadena
Armstrong, Richard C.
Balding, Laurence G.
Johnson, Henrietta M.
Morgan, George E.
Riffenburgh, Ralph S.

Redondo Beach
Gorchenko, Victor A.

Redwood City
Golden, Stanley D.

Richmond
Morgan, Meredith W., Jr.

Riverside
Biegel, Albert C.
Crawford, Walter J.
Mullen, Raymond S.

Sacramento
Kahler, Arthur R.
Wagner, Alfred W.

Salinas
Kraft, Frederick W.

San Bernardino
Quint, J. Harley, Jr.

San Diego
Bloomenthal, John
Merrill, H. Ross
Monsees, Wayne E.

San Francisco
Atkinson, Marshall B.
Barkan, Hans
Barkan, Otto
Bettman, Jerome W.
Borley, W. E.
Campion, George S.
Colyear, Bayard H., Jr.
Cordes, Frederick C.
Crawford, Joseph W.
Dellaporta, Angelos
Erickson, Olive F.
Fine, Max
Foerster, Helenor C.
Friedman, M. Wallace
Goodwin, Rufus C.
Hall, Thomas G.
Harrington, David O.
Henry, Margaret
Hogan, Michael J.
Hosford, George N.
Jakobovits, Rafael
Jampolsky, Arthur J.
Kelley, Robert R.
Kimura, Samuel J.
Lachman, George S.
Loeb, Donald R.
McEwen, William K.
Miller, Miriam
Pischel, Dohrmann K.
Rodin, Frank H.
Rosenberg, Alan J.

Rubin, Melvin L.
Shaffer, Robert N.
Suran, Anita A.
Tamler, Edward
Tour, Robert L.

SAN JOSE
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Thygeson, Phillips
Vaughan, Daniel G., Jr.

SAN LEANDRO
Smith, Jaroud B., Jr.

SAN MATEO
Abernethy, Rodney E.
Westsmith, Richard A.

SAN PEDRO
Zugsmith, George S.

SAN RAFAEL
McBain, Earle H.
Smith, Taylor

SAN YSEDRO
Flynn, Vincent P.

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Anderson, Thomas W.
Forrest, Robert L.

SANTA BARBARA
Loutfallah, Michel

SANTA CRUZ
Trolan, Howard

SANTA MARIA
Mesirow, M. E.

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Bierman, Edward O.
Sinskey, Robert M.
Smithson, Robert A., Jr.

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Aiken, Samuel D.
Lightfoot, Vernon F.

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Goodman, Sanders A.
Roberts, James E.

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Powell, James R.

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Madeley, H. Randall

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BOULDER
Golden, Bruce

COLORADO SPRINGS
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Wetzig, Paul C.

DENVER
Sweta, Edward J.
Tyner, George S.
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Frenkel, Henry H.

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Cutler, Morton

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Walsh, Rita

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Kent, Louis R.
Kresca, Frank J.

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Bishop, Harding E.
Brown, E. V. L.
Cowen, Jack P.
Cushman, Beulah
de Francois, Walter
Friedman, Max B.
Guibor, George
Haas, Joseph S.
Henry, Marvin D.
Hoeltgen, Maurice M.
Holland, Malvern C.
Hughes, William F., Jr.
Hurwitz, Paul
Iser, Gilbert
Klien, Bertha A.
Kronfeld, Peter C.
Lieberman, Howard L.
Mann, William A.
Matusak, Lucian R.
Meyer, Samuel J.
Nethercut, Glenway
Newell, Frank W.
Pearlman, Maurice D.
Puntenney, Irving
Pushkin, Edward A.
Riesen, Austin H.
Roper, Kenneth L.
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Schall, Samuel M.
Scheffler, Milton M.
Schultz, Abraham
Shapira, Theodore M.

Shoch, David E.
Skowron, John J.
Snydacker, Daniel
Sternberg, Paul
Stillerman, Manuel L.
Swiontkowski, Stanley D.
Tepper, Norman N.
Vail, Derrick
Van Wien, Stefan
Vickery, Robert D.
Wescott, Virgil
Zekman, Theodore N.

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Lhotka, F. F.

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Soper, Gail R.

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Sarnat, Leonard A.

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Gallardo, Edward

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Weston, Charles L.

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Good, Palmer
Kirk, Harold Q.
Theobald, G. D.

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Wyman, George J.

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McReynolds, William U.

ROCKFORD
Pritikin, Roland I.

SKOKIE
Monninger, Robert H. G.

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Weisbaum, M. Byron

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McDonald, James E.

WILMETTE
Brown, David V. L.

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Heath, Gordon
Hofstetter, Henry W.

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Wesson, Thomas W.

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Rothberg, Maurice

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Young, Robert L.

HAMMOND
Kuhn, Hedwig S.

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Harger, Robert W.
Larkin, Bernard J.
Mann, Mortimer
Masters, Robert J.
Rutherford, Cyrus W.
Schlaegel, Theodore F., Jr.
Wilson, Fred M.

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Philbrook, S. S.

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Morris, Jean W.

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Burnett, Arthur

SOUTH BEND
Cassady, J. Vernal

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CARROLL
Sullivan, John V.

CEDAR RAPIDS
Noe, Carl A.

CLINTON
Weih, Elmer P.

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Downing, Arthur H.
Lambrecht, Paul

FORT DODGE
Kluever, H. C.

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Allen, Lee
Arnott, George P.
Blodi, Frederick C.
Braley, Alson E.
Burian, Hermann M.
Janes, Ralph G.
Leinfelder, P. J.
Richards, Richard D.
Walker, Glenn L.
Whiteford, Robert D.
Wise, Arthur C.

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Wolfe, Otis D.
Wolfe, Russell M.

OSKALOOSA
Atkinson, George S.

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Phelps, Gardner D.

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Bribach, E. J.

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Fleming, David G.

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Keeney, Arthur H.
MacDonald, Roderick, Jr.
Pfingst, Harry A.
Townes, C. Dwight

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Simmonds, Noel T.

METAIRIE
Holland, Monte G.

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Bahn, Charles A.
Bahn, Gustav C.
Barber, Aeleta N.
Boles, William M.
Byers, Jerome L.
Clark, William B.
Ellis, George S.
Fisher, Earl, Jr.
Haik, George M.
Jimenez, Timoteo
Leckert, Edmund L., Jr.
Mac Donald, Roderick, Jr.
Rosenthal, J. William
Schoel, Robert E.

SHREVEPORT
Gray, Leon F.

MAINE

BANGOR
Osler, Jay K.

LEWISTON
Tchao, Jou S.

PORTLAND
Maier, Paul

WATERVILLE
Dennis, Richard N.
Hill, Howard F.

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Abrahams, Irwin W.
Brumback, Joseph E., Jr.
Duke, James R.
MacLean, Angus L.
Maumenee, Alfred E.
Naquin, Howard
Patz, Arnall
Pogell, Burton M.
Rowland, Louise S.
Stone, Howard H.
Wood, Ronald M.
Woods, Alan C.

BETHESDA
Chalfant, W. Paxson, Jr.
Gunkel, Ralph D.
Hart, William M.
Livingston, Robert B.
Macri, Frank J.
O'Connor, George R.
von Sallmann, Ludwig

CHEVY CHASE
O'Rourke, James F.

SILVER SPRINGS
Goodman, George

MASSACHUSETTS

BOSTON
Allen, Henry F.
Balazs, Endre A.
Beetham, William P.
Boruchoff, Saul A.
Casten, Virgil C.

Chandler, Paul A.
Chisholm, Julian F., Jr.
Cogan, David G.
Devi, Anima
Donahue, Hugh C.
Dunphy, Edwin B.
Futterman, Sidney
Grant, W. Morton
Gunderson, Trygve
Heath, Parker
Jakus, Marie A.
Kern, Harold L.
Kinoshita, Jin H.
Kupfer, Carl
Kuwabara, Toichiro
Liebman, Sumner D.
Lo-Presti, Joseph J.
Merola, Lorenzo O.
Murray, Edward S.
Regan, Charles D. J.
Schepens, Charles L.
Sloane, Albert E.
Snyder, John C.
Verhoeff, F. H.

BROOKLINE
Stone, William

CAMBRIDGE
Wald, George

MEDFORD
Carpenter, Russell L.

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Ryan, William F.

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Corcoran, George B., Jr.
Waugh, Richey L., Jr.

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Broggi, Richard J.
Holzer, William F.
Myers, Roscoe W.
Whitney, Percy T.
Yasona, Elton

MICHIGAN

ANN ARBOR
Alpern, Mathew
Falls, Harold F.
Fralick, F. Bruce
Henderson, John W.
Scharenberg, Konstantin
Smillie, John W.
Wolter, J. Reimer

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DETROIT
Bialik, Michael H.
Burroughs, Roswell G.
Crossen, Robert J.
Davies, Windsor S.
Frey, James L.
Guyton, Jack S.
Johnson, David S.
Kinsey, V. Everett
Lepard, C. W.
Lerner, Leonard H.
Neeb, Walter
Olson, James A.
O'Rahilly, Ronan
Pugh, Howard C.
Reddy, D. Venkat
Ruedemann, A. D.
Ruedemann, A. D., Jr.
Schane, David
Scarney, Herman D.
Schimek, Robert A.
Sugar, H. Saul
Wachtl, Carl
Wehr, Maurice B.

FLINT
Rundles, Walter Z.

JACKSON
McGarvey, William E.
Wholihan, John W.

NILES
Moore, T. Scott

TRAVERSE CITY
Beall, John G.
Weih, Jack E.

YPSILANTI
Petrohelos, Manousos A.

MINNESOTA

DULUTH
Hilding, Anderson C.

MINNEAPOLIS
Fink, Walter H.
Friedman, Harry S.
Halbert, Franz
Hoffman, Walter L.
Kantar, Bruce L.
McCannel, Malcolm A.
Sandt, Karl E.
Schwartz, Virgil J.
Shapiro, Irving
Simonson, Ernst
Walsh, Francis M.
Wendland, John P.

ROCHESTER
Bair, Hugo L.
Benedict, William L.
Henderson, John W.
Hollenhorst, Robert W.
Martens, Theodore G.
Ogle, Kenneth N.
Rucker, C. Wilbur
Sabin, Frederick C.
Sheard, Charles
Stein, Harold A.
Steinmetz, Rodney D.
Winn, William E., Jr.

ST. PAUL
Burch, Edward P.
Grant, Hendrie W.
Monahan, Robert H.
O'Kane, Thomas W.
Strate, Gordon E.

MISSISSIPPI

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Gamble, Lyne S.

GULFPORT
Flagg, G. B.

JACKSON
Johnson, Samuel B.

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Bryan, James H.
Drews, Leslie C.
Freeman, David M.
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Israel, Milton R.

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Stauffer, Harry B.

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McKee, Joseph W.
McLeod, John
May, James W.
Miller, Wade H.
Padfield, Earl G., Jr.
Robison, James T., Jr.
Rufe, John R.
Shaad, Dorothy J.

MEXICO
Rouse, David M.

ST. LOUIS
Alvis, Edmund B.
Becker, Bernard
Bisno, Daniel
Christensen, Robert E.
Cibis, Paul A.
Constant, Marguerite A.
Enoch, Jay M.
Hartstein, Jack
James, William M.
Luedde, Philip S.
Mattis, Robert D.
Miles, Paul W.
Miller, James E.
Moses, Robert A.
Post, Martin H., Jr.
Rosenbaum, Harry D.
Sanders, T. E.
Schwartz, Frederick O.
Shahan, Philip T.
Venable, Howard P.

NEBRASKA

LINCOLN
Paulson, Hubert O.
Thomas, Richard L.

OMAHA
Alliband, George T.
Filkins, John C.
Gifford, Harold
Judd, John H.
Lipp, Frank E.
McIntire, Walden C.
Morrison, W. Howard
Rasgorshek, Robert H.
Swab, Charles M.
Truhlsen, Stanley M.

NEVADA

RENO
Magee, George R.

NEW HAMPSHIRE

BERLIN
Ingalls, Raymond G.

HANOVER
Auten, Hanford L.
Sullivan, Paul B.

NEW JERSEY

ASBURY PARK
Fisher, James A.

ATLANTIC CITY
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BOUND BROOK
Levy, Abram

CAMDEN
Meyer, George P.

EAST ORANGE
Cregar, John S.
Jaeckle, Charles E.

FAIRLAWN
Seligson, Alvin

FAR ROCKAWAY
Goldsmith, Maximilian O.

HACKENSACK
Berke, Raynold N.

HADDONFIELD
Finley, John K.

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Nicholson, F. Peter

LONG BRANCH
Roberts, N. Craig

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Sherman, A. Russell
Sherman, Elbert S.

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D'Amico, Thomas V.
Ehrenfeld, Edward
Lang, Richard E.
Silverstein, Arthur L.

PLAINFIELD
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Abrams, Henry
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SHORT HILLS
Fonda, G. E.

SUMMIT
McAlpine, Paul T.

TRENTON
Murto, Robert E.
Sacks-Wilner, Erwin
Sharbaugh, George B.
Wilner, Arthur S.

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ALBUQUERQUE
Dillahunt, Jack A.
Schonberg, Albert L.

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Bedell, Arthur J.
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AMSTERDAM
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BAYSIDE
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BINGHAMTON
Landers, Philip H.
Walling, Harry G.
Werner, George B.

BRONX
Burman, Daniel
Fleischner, Alois L.

BROOKLYN
Ajello, Dominick A.
Beery, Edwin N.
Bodian, Martin
Bonadia, Calogero
Cholst, Mortimer
Evans, John N.
Fink, Austin I.
Sanders, T. E.
Grassi, Anthony J.
Levine, George
Levitt, Jesse M.
Mandelbaum, Joseph
Rizzuti, A. Benedict
Rosenberg, Abner S.
Rosenthal, Benjamin C.
Sands, Abraham M.
Schwartz, Bernard

BUFFALO
Addington, Charles H.
Bennett, Arthur L.
Brennan, James W.
Buckheit, Rudolph G.
Cowper, Alexander R.
Fial, Edward A.
Fowler, James G.
Freeman, Sheldon B.
Higgs, Howard H.
Howard, William M.
Jones, W. Yerby
LeWin, Thurber
Luhr, John P.
Naples, Ange S.
Noell, Werner K.
Olmsted, K. Elizabeth P.
Reitz, Herbert R.
Schopp, Robert C.
Smallen, Benjamin

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Hoffman, Parker M.

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Kellerman, Leo D.

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Cole, John G.
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Constantine, F. H.
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Feinstein, Robert R.
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Foote, Franklin M.
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Friedman, Benjamin
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Givner, Isadore
Goodside, Victor
Gordon, Dan M.
Gorin, George
Guy, Loren P.
Halberg, G. Peter
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Kahan, Edmund
Kara, Gerald B.
Katzin, Herbert M.
Kaufman, Ira H.
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Knapp, Arthur A.
Knapp, Phillip
Koppel, Zoltan I.
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Krohn, David L.
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Levene, Ralph Z.
Linksz, Arthur
Lisman, Jack V.
Locatcher-Khorazo, Deborah
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Lubkin, Virginia

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Payne, Brittain F.
Perera, Charles A.
Posner, Adolph
Proctor, Malvin
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Roberts, Bernard A.
de Roetth, Andrew, Jr.
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Sauer, John J.
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Schachne, Lewis
Schlossman, Abraham
Schneider, Julius
Schultz, Sigmund
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Sykowski, Peter S.

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Katz, I. Herbert
McGraw, James L.
Marlow, Searle B.

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Valone, Richard J.

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WANTAGH
Esbin, Leo

WARSAW
Fountain, Newland W.
Leachman, John W.

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Atkinson, Walter S.

WHITE PLAINS
Duncan, James A.

NORTH CAROLINA

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Odom, Robert E.

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Byerly, Baxter H.

CHARLOTTE
Ghent, Thomas D.

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Anderson, Banks
Draheim, Jerry W.
McPherson, Samuel D., Jr.
Stocker, Frederick W.

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Bizzell, James W.

WINSTON-SALEM
Holt, Lawrence B.
Roberts, R. Winston

NORTH DAKOTA

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Prochaska, Leonard J.

MINOT
Kohl, Darwin L.

OHIO

AKRON
Krichbaum, Franklin M.

ALLIANCE
King, George L.

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Abrahamson, Ira
Abrahamson, Ira H., Jr.
Asbury, Taylor
Ascher, Charles K. W.
Brown, Albert L.
Freemond, Alan S.
Ginsberg, Joseph
Kemper, Robert A.
Lyle, Donald J.
Nelson, Robert C.
Reid, Horace W.
Sakler, Barnet R.
Schrimpf, Cyril E.

CLEVELAND
Adams, Elliot Q.
Ballintine, Elmer J.
Bennett, James E.
Binder, Hertha F.
Binder, Rudolf F.
Brandwan, Samuel R.
Budd, Francis X.
Chamberlain, W. P., Jr.
Farkas, Tibor G.
Gans, Jerome A.
Gans, Morris E.
Guth, Sylvester K.
Hardesty, Hiram H.
Hare, James
Harvey, Elmer F.
Hertner, John E.
Johnson, Lorand V.
Kazdan, Philip
Kennedy, Roscoe J.
Keyes, John E. L.
Kirk, Robert C.
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